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**Swine lairage pen cleaning and disinfecting: effects on prevalence and antimicrobial
sensitivity patterns of *Salmonella enterica* isolates**

by

Peggy Lynn Schmidt

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Veterinary Preventive Medicine

Program of Study Committee:
Annette O'Connor, Co-major Professor
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Iowa State University
Ames, Iowa
2004

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Graduate College
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This is to certify that the master's thesis of
Peggy Lynn Schmidt
has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

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DEDICATION

This thesis is dedicated to my family, both close and extended. You have always been supportive of my decisions to continue in educational endeavors and for that I am eternally grateful. To my parents, Don & Jean Moats; thanks for your years of encouragement, you always said I could do whatever I wanted to do (I bet you never thought it involved being a professional student). To my in-laws, Donald & Darlene Schmidt; thanks for your patience in listening to me babble about things that only a grad student could enjoy and always acting like it excited you just as much. To my husband Gary; thanks for your lifetime of patience and encouragement. I could never have accomplished all I have without you. To my daughter Emma; it looks like mommy will finally be done with school. Thanks for your pictures on my office walls, enthusiasm for cows and piggies, and endless hugs and kisses.

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ABSTRACT

The purpose of this study was to determine the effects of lairage pen cleaning and disinfecting (C & D) procedures on prevalence of *S. enterica* infected swine at slaughter and antimicrobial resistance patterns of residual *S. enterica* isolates.

A series of four field trials were conducted in a large Midwestern abattoir. Each trial consisted of a cleaned (alkaline chloride detergent) and disinfected (H_2O_2 + peracetic acid sanitizer) pen (treated) and a control pen. Intestinal lymph nodes, cecal contents, and fecal contents were collected from 45 pigs in each pen at harvest and cultured for *S. enterica*. In all trials, cleaning and disinfection reduced the prevalence of *S. enterica* positive floor swabs in the treated pen ($p < 0.05$). In Trial 1, no significant difference in prevalence of *S. enterica* in pigs between treatment and control groups occurred. In Trials 2 and 3, prevalence of *S. enterica* was higher in pigs from treated pens vs. pigs from control group pens (91% vs. 40%, $p < 0.0001$ and 91% vs. 24% $p < 0.0001$ respectively). In Trial 4, prevalence of *S. enterica* was lower in pigs from treated pens vs. pigs from control pens (5% vs. 42%, $p < 0.0001$).

Pen swabs were collected from each treated lairage pen before and after application of C & D procedures. Antimicrobial resistance of recovered *S. enterica* isolates was determined using Kirby-Bauer disk diffusion test methods and results reported as sensitive, intermediate, or resistant to tested antimicrobials. Recovered isolates clustered into 10 resistance patterns. Prevalence of *S. enterica* positive pen swabs and resistance pattern diversity decreased after C & D procedures. C & D procedures appeared to be non-selective in overall residual *S. enterica* resistance patterns.

Swine lairage pen C & D procedures consistently reduce, but do not eliminate residual *S. enterica* organisms within lairage pens. However, reductions in prevalence of *S. enterica* in lairage pens do not translate into reductions in prevalence if *S. enterica* infected pigs held in those pens. If C & D procedures are used in lairage situations, the impact on antimicrobial resistance of remaining isolates appears to be minimal.

CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

This thesis is organized into five chapters. Chapter 1 begins with a description of how this thesis is organized. Following the thesis organization is a brief review of commonly used disinfectants including concerns regarding their use in food production areas. Originally this review of disinfectants was included in the literature review, but subsequently removed. I believe this section offers readers a brief review of mechanisms of action necessary to understand how these compounds affect bacterial organisms, including *S. enterica*. Chapter 1 concludes with a statement of the problem addressed by the included research.

Chapter 2 is a literature review designed for publication. This review utilizes a systematic review format which is becoming more popular in human medical literature. This format not only facilitates an understanding of current subject knowledge but also allows critical appraisal of each study and its results. Chapter 3 is the first research paper from this Masters project. It addresses the ability of lairage pen cleaning and disinfection to reduce the amount of *S. enterica* present in the lairage pens as well as the prevalence of *S. enterica* infected pigs in both clean and dirty pens. Chapter 4 is the second research paper from this Masters project. In this study, *S. enterica* organisms isolated from lairage pen swabs are subjected to Kirby-Bauer disk diffusion tests to determine resistance to 12 chosen antimicrobials. Comparisons are made between before and after cleaning and disinfecting pen samples. Chapter 5 presents general conclusions drawn from the research described in this thesis.

Review of Disinfectants

Mechanisms of action

Mechanisms of antimicrobial action exhibited by specific disinfectants can be similar within and between types of disinfectant. Common types of disinfectants used in animal production systems are listed in Table 1.1. Brief descriptions of four common antimicrobial mechanisms follow.

Reactive oxygen species

Formation of reactive oxygen species, such as hydrogen peroxide, superoxide anion, hydroxyl radicals and oxygen singlets, in response to halogen or peroxygen disinfectant exposure lead to exhaustion of antioxidant defenses and lethal DNA damage (Dukan *et al.* 1999). Oxidation of sulfhydryl groups, by disinfectant derived free radicals, leads to inactivation of cytosolic sulfhydryl dehydrogenase enzymes, decreasing cellular metabolic activities and reducing cell viability (Leyer & Johnson 1997). Loss of succinate-dependent respiration is closely associated with halogen or peroxygen disinfectant targeting and inactivation of iron-sulfur centers in succinyl dehydrogenase enzymes (Hurst *et al.* 1991).

Lipids within cell membranes react with hydroxyl radicals initiating lipid peroxidation. Lipid peroxidation alters membrane permeability and disrupts membrane bound proteins which affects nutrient transport and ATP production, as well as leading to further free radical formation. Base and sugar groups of nucleic acids are targeted by reactive oxygen species creating single and double stranded breaks within DNA and single strand RNA breaks (Cabiscol *et al.* 2000). This damage leads to bacterial cell death.

Cytoplasmic membrane disruption

Alcohols, aldehydes, biguanides, cationic agents, and phenols are often referred to as the “membrane-active” disinfectants as they disrupt the cytoplasmic membranes of microorganisms to induce cell death (Russell 2001). Membrane disruption leads to leakage of potassium and low molecular weight materials that alter the cell permeability and interrupt nutrient transport. The type and amount of interaction of the disinfectants with the

cytoplasmic membrane components determines the amount of membrane disruption and subsequent cellular dysfunction and death (McDonnell & Russell 1999).

Disruption of proton motive force

Proton motive force consists of a difference in H^+ concentration (proton gradient) and electrical potential across the cell membrane and is responsible for ATP synthesis and nutrient transport. Organic acids cause disruption of the proton gradient and inhibition of ATP synthesis leading to cell death. Some phenols and cationic agents also disrupt the proton motive force in microorganisms (Maillard 2002).

Protein coagulation & denaturation

Aldehydes, alcohols, biguanides, cationic agents, and phenols cause coagulation, cross-linking or denaturation of cytoplasmic proteins (Scott & Gorman 2001). Base and sugar groups of nucleic acids are targeted by reactive oxygen species (halogen and peroxygen agents) creating single and double stranded DNA breaks and single strand RNA breaks (Cabiscol *et al.* 2000). Subsequent loss of enzymatic functions or nucleic acid damage results in cell death.

Efficacy

A comprehensive review of methods for testing efficacy of disinfectants is provided by Cremieux *et al.* (2001). Briefly, efficacy of a disinfectant against *S. enterica* or any microorganism is dependant on environmental factors and factors inherent to the microorganism. Testing and claims of product efficacy involve use of a standard laboratory strain of bacteria grown in an optimum culture environment. Common methods of testing include suspension tests, carrier tests, in-use or field tests, and biofilm tests. Reported efficacy in one testing method, such as suspension tests, does not convey efficacy in another testing method, such as field or biofilm tests as phenotypic differences between cells, even of the same strain, play a large role in potential resistance to disinfectants.

Disinfectant resistance

Intrinsic cellular mechanisms of disinfectant resistance, such as; (i) alteration of disinfectant target site, (ii) increased cellular impermeability, (iii) enzymatic modification or destruction, and (iv) efflux pumps, exist in bacterial populations (Poole 2002). In the case of biofilm bacteria, specific extrinsic properties exist that contribute to a 10-100 fold increase in resistance to antibiotics and disinfectants compared to planktonic bacteria (Gilbert & McBain 2001; Lewis 2001; White & McDermott 2001). These extrinsic properties include (i) reaction-diffusion interaction, (ii) altered protein expression, (iii) phenotypic changes, and (iv) biocide-related apoptosis.

Reactions between disinfectant molecules and biofilm constituents can neutralize disinfectants before diffusion into the deepest layers of the biofilm occur leading to gradients of disinfectant concentration. This process is known as reaction-diffusion interaction (Stewart *et al.* 1998). For example, the killing efficacy of chlorine based disinfectants decreases as the thickness of a biofilm increases (Xu *et al.* 2000). In the case of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* biofilms, diffusion of the hypochlorite (HOCl) is greatly retarded due to reactivity with biofilm constituents while chlorosulfamine, which has a lower capacity for reactivity, demonstrates superior penetration reaching deep biofilm layers eight times faster than alkaline hypochlorite (Stewart *et al.* 2001).

Given that reaction-diffusion interaction at least partially acts as a limiting factor in the activity of highly reactive disinfectants, increasing the concentration of disinfectant rather than exposure time may deplete the ability of a biofilm to react with disinfectant molecules and allow for greater penetration and improved efficacy.

Recent work has identified changes in protein expression of cells within biofilms as another potential source of disinfectant resistance. Phenotypic differences in protein expression can already be detected between 2- and 18-h-old biofilm and planktonic cells (Oosthuizen *et al.* 2002). In the deeper layer of the biofilm, decreasing the availability of nutrients causes up-regulation of DNA-binding protein regulators. These protein regulators create proteins which bind to DNA structures protecting nucleic acids from oxidative damage

as well as increasing synthesis of cellular proteins necessary for biofilm growth and survival (Tremoulet *et al.* 2002b). Cells exhibiting higher degrees of protein up-regulation therefore are less susceptible to the lethal affects of disinfectants such as halogens and peroxygens that utilize hydroxyl radical binding of nucleic acids to induce cell death. Proliferation of cell populations expressing a less-susceptible phenotype leads to the production of a new generation of biofilm bacteria more resistant to disinfectants than the previous generation.

Changes in bacterial dependence on metabolic substrates may change susceptibility to disinfectants. For instance, loss of cellular succinate-dependent respiration through changes in a cell's metabolic pathways, as occurs in *E. coli* and *Pseudomonas aeruginosa*, can create resistance to microbicidal action of chlorine inactivation of iron-sulfur centers in succinyl dehydrogenase enzymes (Hurst *et al.* 1991). Similar mechanisms may occur in *S. enterica*.

Biocide-related apoptosis has been proposed as a means of explaining survival properties of a biofilm following disinfectant exposure. Lewis (Lewis 2000) describes apoptosis as a mechanism of biofilm survivability rather than disinfectant resistance. Survival of the biofilm can be explained by the existence and proliferation of persister cells, those cells surviving following disinfectant exposure. Persister cells may actually not possess intrinsic mechanisms of disinfectant resistance but possess a defect in the ability to carryout a normal programmed cell death response following disinfectant exposure (Gilbert & McBain 2001).

Interactions between multiple species in a biofilm may also affect the ability of a disinfectant to successfully eliminate *S. enterica* or other pathogens from the material surfaces. Biofilm population size increases with increasing species diversity and resistant populations have enhanced the survival of unrelated organisms within the same biofilm (Whiteley *et al.* 2001).

Statement of Problem

Foodborne pathogens are estimated to account for over 76 million cases of illness or disease in the United States annually. From 1988 – 1992, approximately 69% of bacterial

foodborne disease outbreaks reported by the CDC were attributed to *Salmonella enterica* (Bean *et al.*, 1996). In the U.S., *S. enterica* organisms are responsible for over 1.4 million cases of acute gastroenteritis annually with over 16,000 patients hospitalized and over 550 deaths (30% of all deaths attributed to foodborne pathogens) (Morgan *et al.*, 1997).

Estimates for costs associated with foodborne Salmonellosis alone are between \$0.5 and \$2.8 billion annually (Frenzen *et al.*, 1999). *Salmonella* has been implicated in numerous foodborne outbreaks associated with consumption of pork products (Gessner *et al.* 1994, Maguire *et al.* 1993, Narain *et al.* 1989). Recent published work has indicated 3.3 - 9.6% of pork samples from retail outlets and 5.8% of ground pork samples from processing plants are contaminated with *S. enterica* (Duffy *et al.* 2001, Zhao *et al.* 2001). Accounting for flaws in sample collection and sensitivity and specificity problems with diagnostic methods, Berends (Berends *et al.* 1996) estimated the true prevalence of *S. enterica* in primal cuts and retail pork to be closer to 25-30% and in ground pork and pork sausages, 50-55% of product. In order to reduce the potential impact of *S. enterica* related foodborne illness associated with pork, the swine industry has made developing intervention methods to reduce the burden of *S. enterica* in swine a priority.

One of the most commonly recommended intervention methods for reduction of *S. enterica* in livestock production continues to be cleaning and disinfecting animal housing facilities (Du 1999, Hutchinson *et al.* 1999, Wetzell 1998). While this method agrees with the most basic common sense notions about infectious disease, removing or killing infectious organisms, both observational and field trial studies have failed make a definitive association between cleaning and disinfecting procedures and a reduction in the prevalence of *S. enterica* infected pigs (Baum *et al.* 1997, Boes *et al.* 2001, Funk *et al.* 2001, McLaren *et al.* 2001, Oosterom *et al.* 1982, Stege *et al.* 1997). The purpose of this Masters degree project was two-fold. First, could cleaning and disinfecting swine lairage pens reduce the incidence of *S. enterica* positive pigs at slaughter? Second, would cleaning and disinfecting lairage pens affect antimicrobial resistance patterns in the residual *S. enterica* population in those pens? Chapters 3 and 4 of this thesis address those questions.

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Table 1.1: Common disinfectants used in animal production

Type of Disinfectant	Examples	Spectrum of Activity	Antibacterial Mechanisms of Action	Organic Material	pH	Comments
Alcohols	benzyl, ethyl, isopropyl & methyl alcohols	bacteria, fungi, +/- viruses, +/- spores	<ul style="list-style-type: none"> • protein coagulation & denaturation • cell wall disruption • cytoplasmic membrane disruption 	some activity	no effect	<ul style="list-style-type: none"> • bacterial resistance not an issue • volatile at high concentrations • spectrum of activity dependant on concentration and type of alcohol • synergistic effects with QACs, halogens and phenols
Aldehydes	glutaraldehyde, formaldehyde	bacteria, fungi, viruses, spores	<ul style="list-style-type: none"> • cell wall disruption • cytoplasmic membrane disruption • protein, RNA & DNA denaturation 	active	neutral to high pH	<ul style="list-style-type: none"> • generally considered non-corrosive • alkaline solutions rapidly lose efficacy during storage
Biguanides	chlorhexidine	bacteria, fungi, +/- viruses, +/- spores	<ul style="list-style-type: none"> • cytoplasmic membrane disruption • protein coagulation 	some activity	optimum pH range 5 to 8	<ul style="list-style-type: none"> • precipitates in hard or alkaline water • incompatible with organic or inorganic anions
Cationic Agents	quaternary ammonium compounds (QACs)	bacteria, +/- fungi, +/- viruses	<ul style="list-style-type: none"> • cytoplasmic membrane disruption • protein denaturation 	inactive	neutral to high pH	<ul style="list-style-type: none"> • concurrent detergent activities • inactivated by soaps and detergents (anionic) • less effective against gm- than gm+ bacteria • significant resistance in <i>Pseudomonas</i> sp. • may be incompatible with rubber
Halogens	iodine & chlorine compounds	bacteria, fungi, viruses, spores	<ul style="list-style-type: none"> • formation of reactive oxygen species • disrupts bacterial ATP production • cytoplasmic membrane disruption • DNA strand breakage 	inactive	low to neutral pH	<ul style="list-style-type: none"> • effective at low concentrations • chlorine effective in hard water • readily available • generally inexpensive • corrosive to metals • ineffective in presence of ammonia or amino compounds
Organic Acids	acetic, benzoic, lactic & propionic acids	bacteria, fungi, +/- viruses	<ul style="list-style-type: none"> • disruption of proton motive force • uncoupling of electron transport 	active	low to neutral pH	<ul style="list-style-type: none"> • corrosive • slow activity • extensive use as food preservatives
Peroxygens	hydrogen peroxide (H ₂ O ₂), peracetic acid (PAA)	bacteria, fungi, viruses, spores	<ul style="list-style-type: none"> • formation of reactive oxygen species • DNA strand breakage 	H ₂ O ₂ inactive, PAA active	effective in pH range 2 to 10	<ul style="list-style-type: none"> • synergistic effects with Cu⁺⁺, Fe⁺⁺, UV radiation, ultrasonic waves • non-toxic at low concentrations • volatile at high concentrations
Phenol & Phenol Derivatives	cresols, parabens, phenols, xylenols, triclosan	bacteria, +/- fungi, +/- viruses, +/- spores	<ul style="list-style-type: none"> • cytoplasmic membrane disruption • enzymatic inhibition 	some activity	low to neutral pH	<ul style="list-style-type: none"> • found in many essential oils • bacteriocidal at high concentrations • bacteriostatic at low concentrations • less effective against gm- than gm+ bacteria

Table compiled from: Principles and Practice of Disinfection, Preservation and Sterilization, 2nd edition; Russell, Hugo and Ayliffe
 Disinfection, Sterilization, and Preservation, 5th edition; Block, editor

CHAPTER 2. PRE-HARVEST CONTROL OF *SALMONELLA* *ENTERICA* IN PORK PRODUCTION BY USE OF CLEANING AND DISINFECTING ANIMAL FACILITIES

A paper to be submitted to Animal Health Research Reviews

Peggy L. Schmidt, DVM

Abstract

In spite of our efforts, *Salmonella enterica* remains one of the most common food borne infections of humans in the United States and around the world. For reduction of *S. enterica* contamination of pork and pork products, cleaning and disinfecting pig holding facilities (farm or lairage) and transportation equipment is a commonly recommended intervention. In fact, cleaning and disinfecting swine housing and transportation equipment is known to reduce the level of *S. enterica* in these environments, but elimination of all culturable *S. enterica* organisms is rare. Importantly, published studies have actually reported both increased and decreased rates of recovery of *S. enterica* from carcasses of pigs housed and transported in cleaned and disinfected environments. In this paper, published studies addressing the role of cleaning and disinfecting animal facilities in reducing the pre-harvest prevalence of *S. enterica* in pigs are reviewed with the objective of evaluating if and how these procedures may contribute to the reduction of food borne illness in humans attributable to *S. enterica* from pork.

Section 1

Introduction

In the United States, *Salmonella enterica* is annually associated with over 1.4 million human cases of acute gastroenteritis leading to 16,000 patient hospitalizations and more than 550

deaths (Morgan *et al.*, 1987). Annual costs associated with *S. enterica* gastroenteritis in the U.S. populace are estimated at \$0.5 to \$2.8 billion (Frenzen *et al.*, 1999). Recent estimates of *S. enterica* contamination in pork products range from 25-30% of primal cuts to 50-55% of ground pork product (Berends *et al.*, 1996). Because of the incidence and associated disease costs in humans, the frequency of *S. enterica*-contaminated pork, and the high profile nature of cases of foodborne disease in the U.S., the pork industry is working actively to reduce the prevalence of *S. enterica* in pigs and pork.

In pigs, *S. enterica* infection can occur at any point from farm to abattoir. Similarly, *S. enterica* contamination of pork can occur at any point from abattoir to consumer. Cleaning and disinfecting protocols are commonly recommended for *S. enterica* control in the pre-, peri- and post-harvest environments. Published literature describing efficacy of cleaning and disinfecting (C & D) of animal facilities as a method for reducing the pre-harvest prevalence of *S. enterica* in pigs will be reviewed in this paper.

This review is divided into four sections. Section 1 introduces the subject of this paper as well as describing review methods and definitions used in the context of this review. Section 2 summarizes *S. enterica* ecology in the micro- and macroenvironment of pig production. For a complete review of *S. enterica* ecology, readers are referred to “Salmonella in Domestic Animals” by Wray and Wray (2000). An understanding of *S. enterica* ecology is necessary for the following discussion the reviewed papers in section 3. Section 3 reviews nineteen papers which address C & D procedures as a means of reducing *S. enterica* in pig production facilities. Results are summarized for papers based on study design. Individual papers are then evaluated based on study design and research methodologies. Tables 2.1 summarize results of all included papers. Finally section 4 discusses conclusions drawn from the reviewed papers.

Review methods

Databases used to obtain potential papers included: AGRICOLA, BeastCD, CAB Abstracts, PubMed, and VetCD with the last search completed on May 9, 2003. Potential papers were selected based on language of publication (English) and database keyword search. Keyword search terms used included, “Salmonella” and “disinfection” or “cleaning”. Using the keyword search terms, 484 citations of potential papers were identified. Titles of the 484 citations were examined for language referring to swine, pigs, hogs, pork, lairage, abattoir, slaughter, or other pig production terms.

Abstracts of potentially relevant papers were retrieved. Full text papers were retrieved if abstracts referred to cleaning, or cleaning and disinfecting animal holding and/or transport facilities as well as the presence of *S. enterica* in those facilities or prevalence of *S. enterica* in animals held in those facilities. When abstracts were unavailable, full text papers were retrieved and evaluated for similar terms.

Additional papers were found through examination of proceedings from the International Symposiums on the Epidemiology and Control of *Salmonella* and Other Food Borne Pathogens in Pork as well as reference sections of already selected papers. In total, nineteen papers identified from listed databases, referenced papers, published theses and conference proceedings were included in this review.

Definitions

Definitions of terms used to describe cleaning, disinfection and stages of pig production differ between individuals based on experience and previous usage. To avoid confusion amongst readers, a list of definitions as used in this review is included.

Biocide: An agent that kills living organisms; both macro and microorganisms.

Cleaning: The chemical or physical removal of visible debris; includes soil, blood, and protein substances.

Disinfectant: An agent that kills, inhibits, or prevents growth of microorganisms on inanimate objects.

Disinfecting/Disinfection: The destruction of pathogenic and other microorganisms by chemical or other means.

Finishing: The final feeding period of pigs when attain market or slaughter weight is attained. Also commonly referred to as fattening. Used as “finishing pig,” “finishing barn”, or “finishing phase”

Lairage : (n) The animal holding facilities at the abattoir or slaughter plant. (v) The process of holding animals in pre-slaughter holding facilities at the abattoir.

Trailer: Any transport vehicle which moves pigs between facilities. Also commonly referred to as a truck or lorry.

Section 2

S. enterica ecology in microenvironment

In order to grow and proliferate, *S. enterica* and other bacteria seek to attach to solid surfaces which provide themselves and their daughter cells with a nourishing environment. After initial attachment to a solid surface, bacteria replicate and attach to one another to form a single or multi-species colony of bacteria. A cement-like attachment of bacteria to each other and to the solid surface occurs as bacteria secrete extracellular components, mainly fimbriae and a fibrinous or globular exopolysaccharide glycocalyx. The combination of bacteria and extracellular components form a negatively charged organic matrix in which debris and nutrients become trapped. This mass of bacteria, debris, and extracellular material held to a solid surface is known as a biofilm (Zottola, 1994).

Once biofilm formation is initiated, phenotypic changes occur within the bacteria which constitute it (Carpentier and Cerf, 1993). Initially, bacteria closest to the liquid-solid or air-

solid interface of a biofilm maintain a high rate of growth leading to addition of new bacteria to the biofilm surface. Then bacterial growth rates decrease as bacteria become farther removed from the liquid or air surface interface. Therefore as biofilms thicken, the deepest cells enter a state of quiescence requiring only minimal nutrients for survival. However, if adequate nutrients fail to reach the deepest biofilm layers, bacterial death occurs. Bacterial death produces abnormally weak areas of adhesion with detachment and dispersion of portions of biofilm into the environment. In contrast, surface detachment and dispersion of daughter cells is normal and must occur in order to ensure bacterial survival (Kumar and Anand, 1998). Transportation of detached surface bacteria to new locations by fluid or physical means restarts the biofilm formation process (Stoodley *et al.*, 2001).

Experimentally, *S. enterica* biofilm formation occurs within hours of exposure to a solid surface. *S. enterica* biofilms have been established on surface materials commonly used in animal production facilities including chlorinated polyvinyl chloride (CPVC) (Jones and Bradshaw, 1996), plastic, cement, and stainless steel (Joseph *et al.*, 2001). However, *S. enterica* often exist in a planktonic or free-floating state in nutrient-rich broth in the laboratory setting. This planktonic state influences the phenotypic properties exhibited by the bacteria including metabolic rates, nutrient requirements, and susceptibility to biocides, including disinfectants. Testing regarding the efficacy of disinfectants is performed on planktonic cell phenotypes. Biofilm cell phenotypes may translate into significant differences in the efficacy of disinfectants in a field trial setting.

***S. enterica* ecology in pre-harvest pig production macroenvironment**

S. enterica are commonly recovered from finishing pigs in the pre-harvest environment. In a 1995 study, 6% of finishing pens in the United States tested positive for *S. enterica* on fecal culture with 38% of tested farms identified with at least one positive pen sample (Anonymous, 1997). Others have reported fecal isolation of *S. enterica* from 2-50% of finishing pigs prior to transport to abattoirs (Barber *et al.*, 2002, Davies *et al.*, 1999).

Fecal isolation of *S. enterica* from finishing pigs has been associated with; presence of cats, mice, birds, and flies in finishing facilities, contaminated feedstuffs, housing on dirt floors, more than 2 people at a finishing site daily, contaminated clothing/boots moving between pens or barns, and lack of farm and/or employee hygiene (Barber *et al.*, 2002, Funk *et al.*, 2001a, Kranker *et al.*, 2001). Factors associated with high seroprevalence rather than fecal isolation of *S. enterica* include; poor farm hygiene practices, use of contaminated feedstuffs (pelleted, home-mixed, and/or liquid feed) and moderate to large herd size (Berends *et al.*, 1996, Kranker *et al.*, 2001, van der Wolf *et al.*, 2001).

To date, interventions designed to reduce the presence of *S. enterica* organisms in farm environments have shown little success. In early weaned pigs, vaccination, acidification of drinking water, and administration of egg-yolk specific immunoglobulins have been ineffective in reducing occurrence *S. enterica* infection in mesenteric lymph nodes (MLN) or fecal samples (Letellier *et al.*, 2000). As a means of achieving and maintaining high herd health and efficient performance, segmentation of production phases into multiple sites has become commonplace in pig production (Harris, 1988). Unfortunately multi-site production has failed to make a significant impact on reducing the prevalence of *S. enterica* in pig production.

Showing some benefit, bambarmycins administered for at least 14 days prior to and 14 days after infection have reduced carriage of *S. enterica* in MLN samples (Letellier *et al.*, 2000). Similarly fructooligosaccharides (FOS) administered orally to weaned pigs also show promise in reducing fecal shedding of *S. enterica* organisms (Letellier *et al.*, 2000, Nemcova *et al.*, 1999). Administration of oral sodium chlorate to weaned pigs prior to *S. enterica* challenge can also significantly reduce the concentration of *S. enterica* organisms in cecal contents within 24 hours after challenge (Anderson *et al.*, 2001). In pig rations, fermented pig feed reduces survivability of *S. enterica* organisms by reducing environmental pH below

4.5. However, study results remain inconsistent in the ability of fermented feeds to reduce the recovery of *S. enterica* in pigs fed those diets (van Winsen, 2001; van der Wolf *et al.*, 2001).

Denmark currently uses different transport, lairage, and slaughter procedures for high and low *S. enterica* seroprevalence herds as interventions for reducing *S. enterica* in pork with a degree of success. However, utilization of farm level interventions is also part of the Danish broad industry based system of *S. enterica* reduction. Therefore, it is not possible to differentiate components of the program that can be attributed to “transport” and “lairage” or to “farm” (Nielsen, 2001, Alban *et al.*, 2002, Mousing, 1997).

Strong evidence suggests that not all *S. enterica* entering the abattoir in pigs can be attributed to the farm of origin. Increasing prevalence of *S. enterica* in pigs frequently occurs between on-farm and abattoir facilities (Berends *et al.*, 1996, Hurd *et al.*, 2001, Hurd *et al.*, 2002, McKean *et al.*, 2001, McKinley *et al.*, 1980).

Hurd (Hurd *et al.*, 2002) reported a significant increase in *S. enterica* recovery from pen mates slaughtered at the abattoir vs. those slaughtered on-farm. On farm, *S. enterica* was recovered from 5.3% of pigs, however, in pigs exposed to transport and lairage, *S. enterica* was recovered from 39.9% of pigs. Differences between on-farm and abattoir recovery rates can be partially explained by recent studies which demonstrated recovery of *S. enterica* from distal ileum and cecal contents of naive pigs within 30 minutes of exposure to *S. enterica* contaminated environments (Hurd *et al.*, 2001). Additionally, an increased rate of bacterial translocation, movement of bacteria from gut lumen into intestinal tissues, in response to animal transportation may also contribute an increased prevalence of *S. enterica* in pigs at the abattoir (Seidler *et al.*, 2001).

Section 3

In total, nineteen papers identified from listed databases, referenced papers, published theses and conference proceedings were included in this review. These papers address C & D of pre-harvest facilities, both holding and transport, as an intervention method to reduce environmental contamination of *S. enterica* and/or prevalence of *S. enterica* in pigs. The ultimate goal of such interventions is to reduce the amount of *S. enterica* entering the abattoir and in turn reduce the incidence of human Salmonellosis attributable to consumption of pork products.

Experimental studies

Experimental studies differ from epidemiological studies due to the ability of researchers to control all extraneous factors that may affect the outcome of interest. Use of a reference group, allocation of subjects, and control over application of the intervention occur in experimental studies. In practicality, this control of the study environment reduces or eliminates bias so differences in resulting outcomes are attributed in whole to the applied intervention. During keyword search of literature, no experimental studies involving *S. enterica* and C & D in pre-harvest environment of pig production were identified. However, experimental studies regarding efficacy of disinfectants on common food-contact surfaces and in pre-harvest poultry production were identified. Due to similarities between surfaces utilized in food processing facilities and poultry production and surfaces utilized in pig production, four studies involving laboratory evaluation of efficacy of disinfectants in eliminating *S. enterica* are discussed in this review.

Three experimental studies (El Assaad FG *et al.*, 1995, El Assaad FG *et al.*, 1990, Ramesh *et al.*, 2002) utilized artificial contamination of poultry transport crate surfaces with known strains of *S. enterica* to assess efficacy of various C & D procedures or products. Tested materials included galvanized steel, fiberglass and wood samples. Two of these studies (El Assaad FG *et al.*, 1995, El Assaad FG *et al.*, 1990) demonstrated disinfectant efficacy to be

dependent on water temperature, type of disinfectant, concentration of disinfectant and type of material. Galvanized steel surfaces were most easily decontaminated followed by fiberglass surfaces. Wood surfaces demonstrated residual *S. enterica* contamination under all disinfectant concentrations and water temperatures. In the third experimental studies (Ramesh *et al.*, 2002), only three of thirteen tested disinfectants successfully eliminated biofilm populations of *S. enterica* organisms on galvanized steel surfaces. All three effective disinfectants were halogen based disinfectants.

A fourth experimental study (Joseph *et al.*, 2001) evaluated halogen based disinfectants against *S. enterica* biofilms on common food-contact surfaces; plastic, cement and stainless steel. These tested surfaces are commonly used in pig production and lairage facilities as well as transportation equipment. Reduction in recoverable *S. enterica* on these surfaces was both concentration and time dependant. Only the highest of four tested concentrations of disinfectant eliminated all *S. enterica* biofilm populations. Comparatively, planktonic *S. enterica* organisms originating from the same test strains were sensitive to all disinfectants at significantly lower concentrations and lower contact times.

Discussion

These studies suggest disinfectants may be useful in field situations however confounders such as presence of competing bacteria, multi-species biofilms, and different serotypes of *S. enterica* may limit extrapolation of laboratory outcomes to field situations. For example, these experimental studies used homogenous populations of *S. enterica* organisms, while pig holding facilities and transportation equipment carry numerous bacterial species including multiple serotypes of *S. enterica*.

Descriptive studies

Two descriptive epidemiological studies met criteria for inclusion in this review. One study examined C & D in the on-farm environment (Pedersen, 1997) while a second study examined C & D in transportation equipment and lairage pens (Oosterom *et al.* 1982).

On-farm environment

The single on-farm descriptive study (Pedersen, 1997) classified *S. enterica* seropositive swine farms as either “high” or “low” prevalence herds. The study objective was to reduce *S. enterica* sero-prevalence in finishing pigs in “high” (n=6) and “low” (n=13) seroprevalence herds through consistent application of C & D procedures in conjunction with general hygiene and management improvements. With consistent C & D procedures, “high” seroprevalence herds consistently lowered herd prevalence of *S. enterica* seropositive animals. Results in “low” seroprevalence herds were inconsistent with seroprevalence decreasing and increasing in different herds over the 12-month study period. This study suggests the greatest benefit of C & D procedures occurs when *S. enterica* seroprevalence is high. However, another possible conclusion could be that farms with a low seroprevalence already utilize adequate C & D procedures accounting for the lack of similar impact found in high seroprevalence herds

Transportation and lairage environment

One descriptive survey (Oosterom *et al.*, 1982) explored C & D procedures in transport and lairage phases of pig production. Extensive C & D of trailer and lairage facilities did not prevent recovery of *S. enterica* organisms on four separate occasions. Furthermore, *S. enterica* serotypes cultured from pig tissues at slaughter were comparable to on-farm, trailer, and lairage exclusive serotypes.

Discussion

Descriptive studies provide poor evidence of efficacy and serve as hypothesis generating tools. Descriptive studies include case reports, case series, and surveys. Lack of control groups prevents comparisons between case and control animals and therefore prevents associations between intervention exposure and *S. enterica* recovery to occur. This lack of comparison leads to limitations in conclusions drawn from the data. The guarded interpretation from the above studies is that C & D appeared to be ineffective at eliminating *S. enterica* from the environment and similarly appeared ineffective in consistently reducing the prevalence of *S. enterica* in pigs.

Another major disadvantage in the Oosterom study (Oosterom *et al.*, 1982) is lack of pre-C & D pen sampling. Pre-C & D sampling would have allowed evaluation of reductions in *S. enterica* contamination following C & D procedures. Without pre-C & D sampling, interpretation of efficacy of C & D procedures in reducing prevalence of culturable *S. enterica* organisms in pens as pens/ trucks/ lairage may have been negative prior to disinfection cannot occur.

Field trials

Field trials are analogous to clinical trials in design. These studies evaluate an intervention against a control or reference group in their natural environment. Similar to experimental studies, use of a reference group, allocation of subjects, and control over application of the intervention occur in field trials. While researchers have some degree of control, certain aspects of the environment cannot be controlled and may create bias within study results.

Nine field trial studies met inclusion criteria for this review; four involving use of C & D procedures in on-farm environments and five in transport and lairage environments. Efficacy of C & D procedures in these studies was evaluated in two ways. First, did C & D procedures reduce or eliminate *S. enterica* populations in facilities? Second, did C & D

procedures reduce or eliminate prevalence of *S. enterica* infection in pigs housed in those facilities? For studies addressing the second question, *S. enterica* infection was determined by culture (feces or tissues) or serology (presence or amount of circulating *S. enterica* antibodies).

On-farm environment

Four field trials concerning C & D procedures in farm facilities met criteria for inclusion in this paper. With respect to pen prevalence of *S. enterica*, one study did not measure pen prevalence (Dahl *et al.*, 1997), two studies reduced pen prevalence (Oosterom *et al.*, 1982, McLaren *et al.*, 2001) and one study eliminated residual *S. enterica* in pens (Oosterom and Notermans, 1983). With respect to pig prevalence of *S. enterica*, pigs in C & D facilities had lower prevalence of infection than control pigs in two studies (Oosterom *et al.*, 1982, Oosterom and Notermans, 1983), higher prevalence of infection in one study (McLaren *et al.*, 2001), and pigs remained *S. enterica*-free in one study (Dahl *et al.*, 1997).

The first reviewed field trial differs from the remaining three field trials by utilizing serological evidence, rather than culture, to define prevalence of *S. enterica* infection (Dahl *et al.*, 1997). *S. enterica*-free weaned pigs entering C & D facilities remained seronegative throughout nursery and finishing periods whereas cohorts in cleaned, but not disinfected, facilities raised without strategic movement developed circulating antibodies to *S. enterica*. While thorough C & D of facilities apparently eliminated the transmission of *S. enterica* from previously housed animals to currently housed animals, no measurement of prevalence of *S. enterica* organisms in the housing environment occurred before or after treatment. Conclusions about the ability of C & D to eliminate *S. enterica* in this study are therefore assumed, not proved.

The second field trial (Oosterom *et al.*, 1982) utilized high-pressure water cleaning and formalin fumigation to clean and disinfect a 10-pen “sty.” Prevalence of *S. enterica* in finishing pigs housed in C & D pens was compared to pigs housed in non-C & D pens on the

same premises during finishing. Authors reported a significantly lower prevalence of culturable *S. enterica* during the finishing phase in pigs housed in C & D pens versus non C & D pens in two of three trials. A difference in the third trial was numerically obvious (35% vs 57%), but low sample size likely reduced power in this study and prevented determination of statistical significance. Floor swabs were only taken following C & D procedures in the two trials which demonstrated statistical significance. Residual *S. enterica* was not detected in one of these two trails.

Methods of allocation of pigs into C & D or non-C & D pens were not described creating possible bias within this study. Also, as with Oosterom & Notemans (1993) below, numerous changes in hygiene and biosecurity practices occurred concurrently with C&D procedures potentially confounding study results.

The third field trial (McLaren *et al.*, 2001) compared existing C & D procedures on two farms with a new C & D procedure implemented on a third farm. All farms utilized a continuous flow production system. Existing C & D procedures on Farm 1 and Farm 2 reduced prevalence of *S. enterica* in both farrowing rooms and flat decks. Farm 3, which employed new C & D methods, demonstrated an increased prevalence of *S. enterica* positive pen samples after C & D (0/32 vs. 1/80) in farrowing pens while a reduced prevalence occurred in flat decks.

A second part of the McLaren study reported prevalence of *S. enterica* positive pen samples after C & D procedures as well as the end of production. The prevalence of *S. enterica* positive lymph nodes from pigs at harvest was also reported. Three farms utilizing batch production and formaldehyde disinfection between batches had pen prevalence ranging from 0% to 4.4% positive post C & D samples while pen prevalence at the end of the production cycle ranged from 28.7% to 39.5% positive samples. Lymph node prevalence from pigs in these production systems ranged from 4.4% to 8.9% positive samples.

Unfortunately, concurrent studies on effects of acidification of feed and fermented liquid feed were included in this study without a thorough description of study design to assess potential confounding or correlations between those factors and C & D procedures. No description of other farm management or hygiene procedures were included which could address possible confounding by such measures. While sample sizes were given for both pig and pen data to allow for comparison, no statistical analysis of data was reported. This leaves conclusions from this study more equivalent to those of a case report rather than a field study.

Oosterom & Notemans (1983) compared prevalence of *S. enterica* recovered from weekly fecal culture between pigs housed in “normal” facilities and facilities with improved C & D procedures located on the same farm. Improved C & D consisted of cleaning with high-pressure water and disinfecting with a phenol compound solution and formalin fumigation. Vermin control measures, foot baths, clothing changes, and all-in-all-out production were simultaneously implemented with C & D procedures.

C & D procedures apparently removed culturable *S. enterica* from finishing facilities, however, small sample size (n=13) may have contributed to a low probability of detection. However complete disinfection of facilities was not achieved with 11 of 13 samples remaining positive for *Enterbacteriaceae*. In C & D pigs, prevalence of *S. enterica* during the finishing period was 68% (ranging from 0% to 100% over the five month study period) versus 81% (range: 40% to 100%) in pigs housed in other finishing barns on the same farm during the study period.

While inclusion of a control group allows for comparison of prevalence between experimental and control groups, confounding exists between the association of C & D in facilities and concurrently implemented hygiene and biosecurity measures. Although

elimination of culturable *S. enterica* in finishing facilities appeared successful in this study, small sample size may have prevented recovery of remaining organisms. Other farm level factors, such as presence of positive pigs, rodents, feed, or bedding, may also have contributed to environmental contamination after C & D procedures leading to *S. enterica* infection in pigs housed in C & D pens.

Transportation and lairage environment

Five field trial studies specifically addressed C & D procedures applied to swine transportation vehicles and/or lairage facilities. With regards to environmental prevalence, three studies reduced prevalence of *S. enterica* in facilities (Rajkowski *et al.*, 1998, Swanenburg *et al.*, 2001a, Swanenburg *et al.*, 2001b), one study was unable to eliminate residual contamination (Boes *et al.*, 2001) and one study did not measure pen prevalence (Childers *et al.*, 1977). With regards to pig prevalence, no statistical difference in prevalence of *S. enterica* infection in control and C & D pigs occurred in two studies (Boes *et al.*, 2001, Childers *et al.*, 1977) while a third study was unable to prevent *S. enterica* infection in pigs (Swanenburg *et al.*, 2001b).

To start, Rajkowski (Rajkowski *et al.*, 1998) demonstrated a consistent and significant reduction in overall prevalence of culturable *S. enterica* in trailers following C & D procedures (41.5% pre-C & D vs. 2.7% post-C & D). Elimination of culturable *S. enterica* occurred in 94% of contaminated pens within trailers. Only 2.5% of contaminated trailer pens failed to exhibit a significant reduction in culturable *S. enterica*. Authors indicated types of surfaces tested, calculated sample size and sampling distribution, and provided step by step details of C & D procedures and types of disinfectants used (alkaline detergent and QAC disinfectant). Season was also included as an explanatory variable of interest in this study. Both study design and inclusion of season as an explanatory variable addressed possible confounding in this study and strengthens the reported reductions in *S. enterica* following C & D procedures.

Similarly, following use of an alkaline chloride detergent and QAC disinfectant, Swanenburg (Swanenburg *et al.*, 2001a) significantly reduced prevalence of *S. enterica* in swine lairage pens (79%-89% vs. 24%-25% prevalence). An “improved” procedure was able to further reduce pen prevalence to 10% positive samples.

Although authors in this study “strongly suggest that the lairage, as a result of the high level of contamination with *S. enterica*, can have a significant effect in the number of *S. enterica*-infected pigs at slaughter,” no such data on pig prevalence was reported to support to this statement. Whether data in this study was related to another study reviewed next (Swanenburg *et al.*, 2001b) remains unclear but may indicate the basis for the authors’ statements. Conclusions which may be drawn from this study are the ability of C & D to reduce, but not eliminate, the prevalence of *S. enterica* in lairage pens and an indication improved procedures may promote further reduction.

The second study by Swanenburg (Swanenburg *et al.*, 2001b) addressed C & D procedures in both trailers and lairage pens. Authors noted the inability of truck drivers to eliminate *S. enterica* in four of five trailers after application of C & D procedures, despite knowledge that trailers were to be tested. This failure to eliminate residual contamination of *S. enterica* in trucks became a possible source of infection in *S. enterica*-free/seronegative pigs which tested positive for *S. enterica* at harvest. Pre-cleaning trailer prevalence measurements were not included in this study therefore conclusions regarding the ability of C & D procedures to reduce prevalence of *S. enterica* in trucks cannot be made.

In this same study, C & D procedures were unable to eliminate residual contamination of *S. enterica* in lairage pens. Again, no pre-treatment measurements of pen prevalence were available to determine the ability of C & D procedures to reduce culturable *S. enterica*.

Authors noted C & D procedures were described in a study reviewed above (Swanenburg *et al.*, 2001a). Whether these two studies evaluate the same pen data was unclear.

A similar study (Boes *et al.*, 2001) compared prevalence of *S. enterica* in “clean” and control lairage pens. No statistically significant difference in prevalence of *S. enterica* existed between clean and control pens (26.9% vs. 16.7% respectively). Prevalence of *S. enterica* positive carcass swabs from pigs held in clean and control pens also did not differ significantly (1.7% vs 0.8% respectively). As in other field trials, prevalence of *S. enterica* in pens prior to cleaning was not determined, therefore conclusions cannot be drawn regarding the ability of C & D procedures to reduce, if not eliminate *S. enterica* organisms in lairage pens. Even more critical in this study was lack of a definition of “clean” pen. Authors indicated pens were cleaned but disinfectants were not used. Without a description of cleaning procedures, it is difficult to assess the degree to which cleaning actually occurred or if simply a lack of visible manure was construed as being “clean.”

The final included field trial compared prevalence of *S. enterica* positive samples from carcasses and processed pork product when pigs were transported and held in C & D vs. control trailers and lairage pens (Childers *et al.*, 1977). Trailer C & D procedures utilized a combination of phenol and chlorine disinfectants. Lairage pen C & D procedures utilized a combination of iodine and chlorine disinfectants. No statistical difference in prevalence of *S. enterica* was detected in pigs from C & D or control groups.

As with other included studies, researchers did not sample trailers pre- or post-C & D thereby preventing analysis of the ability of C & D procedures to reduce culturable *S. enterica*. This also prevented determination of whether or not exposure to *S. enterica* differed between treatment and control groups or if exposure of pigs to *S. enterica* occurred at all. Therefore, conclusions that C & D procedures in trailer (or lairage) pens have no effect on prevalence of *S. enterica* in pigs at harvest are inappropriate.

Discussion

Field trials allow for the comparison of prevalence between an exposed and non-exposed group. When properly randomized, these studies can provide strong evidence of causation between exposure and disease (or lack of disease in the case of intervention measures). In the case of C & D procedures and *S. enterica* infection in pigs, lack of randomization at the pig level, poor design, and lack of measurement of important variables negates much of the benefit normally afforded by field trials. No description of the sample or referred population also limits the generalization of results in many of these studies.

Epidemiological observational studies

Nine studies classified as observational studies met inclusion criteria for this review. These studies take advantage of the fact that exposed subjects naturally exist and design studies to take advantage those subjects without artificial exposure. Allocation of subjects and application of C & D procedures occurs as a result of observation rather than intervention by researchers. Observational studies in this review included seven cross-sectional studies, two case-control studies and one cohort study. Four cross-sectional studies addressed C & D procedures in on-farm environments only (Bush *et al.*, 1999, Quessy *et al.*, 1999, Beloeil *et al.*, 1999, van der Wolf *et al.*, 2001). One cross-sectional study examined associations between C & D procedures and seroprevalence of *S. enterica* in exposed pigs in both on-farm and transport environments (Baum *et al.*, 1997). Two cross-sectional studies were directed at C & D procedures in the transport and/or lairage environment only (Bahnson and Fedorka-Cray, 1997, Bahnson *et al.*, 2001). Both the single case-control and cohort studies involved C & D procedures in on-farm environments (Stege *et al.*, 1997, Funk *et al.*, 2001b).

Four of five on-farm cross-sectional studies failed to find significant associations between C & D procedures and prevalence of *S. enterica* in finishing pigs (Bush *et al.*, 1999, Quessy *et al.*, 1999, Beloeil *et al.*, 1999, van der Wolf *et al.*, 2001). All three transport/lairage studies

failed to find significant associations between C & D procedures and prevalence of *S. enterica* in finishing pigs (Bahnson and Fedorka-Cray, 1997, Bahnson *et al.*, 2001, Baum *et al.*, 1997). The single on-farm case-control study found a positive association between C & D procedures and high seroprevalence of *S. enterica* in finishing pigs (Stege *et al.*, 1997). Similarly, the single cohort study identified a positive association between “clean” on-farm pens and high fecal shedding of *S. enterica* (Funk *et al.*, 2001b).

On-farm environment

The first included cross-sectional study (Bush *et al.*, 1999) utilized USDA National Animal Health Monitoring System (NAHMS) Swine '95 data. Variables “general farm hygiene” and “cleaning and disinfection of pens” were not significantly associated with an increased incidence of fecal shedding of *S. enterica* organisms in finishing barn pens through multivariate logistic regression. This study included 6655 samples from 988 pens on 152 operations.

While authors stated survey farms were selected from 16 U.S. states which represented 91% of U.S. hog inventory, actual size of sampled farms was not reported. Sampling bias may have occurred in this study if selected farms did not represent a variety of herd sizes. Recall bias may also have occurred through use of a producer questionnaire to determine exposure.

The second cross-sectional study (Quessy *et al.*, 1999) found no statistical association between good production practices (GPPs), such as “units cleaned and disinfected after each lot or every year” and “loading area cleaned and disinfected after each use,” and prevalence of *S. enterica* shedding finishing pigs. Although results were similar to Bush *et al.*'s NAHMS's based study, this study included a small number of farms (n=28) with a small sample size per farm (5 samples per pen, 5 samples per farm). Again, herd size was not reported in this study. Due to small sample size, the lack of association between GPPs and C

& D procedures in this study may have been due to lack of power rather than a true lack of difference between the prevalence of *S. enterica* shedding finishing pigs on farms.

Beloeil (Beloeil *et al.*, 1999) reported a similar lack of association between “cleaning with a detergent” and fecal shedding of *S. enterica* in pigs during the finishing phase of production. Although 69 farms were used, a small sample size within each farm (n=14) decreased the power of this study to detect small differences between farms. Interestingly “duration of the period while rooms were ‘clean and empty’ between two successive batches” of less than one day was associated with high incidence of fecal shedding of *S. enterica* (OR=5.1).

As with previously discussed studies, included herd size was not indicated and exposure was based on a producer questionnaire leaving room for bias in study results. Also, all 69 farms included in this study were affiliated with five pig production or feedstuffs companies. Large production companies often have uniform procedures implemented throughout production systems and could introduce a high degree of correlation between farms. This possibility was not addressed in the study analysis or discussion.

The fourth cross-sectional study (Baum *et al.*, 1997) examined C & D procedures in both farm and lairage environments. Authors were unable to identify a significant association with “cleaning between groups” or “disinfecting between groups” and seroprevalence of *S. enterica* in finishing pigs. Lack of other biosecurity measures, such as “no boot change” and “transport personnel in building” were, however, associated with a higher seroprevalence of *S. enterica*.

Although initially 9,145 serum samples from 267 groups were included in this study, risk factors could only be evaluated for 89 groups and an unknown number of individual pigs. Again, possible recall bias occurred through use of a producer questionnaire to determine C

& D exposure status. Stratification of results by season addressed possible confounding due to seasonal variance in shedding of *S. enterica* adding strength to the study results.

Only one cross sectional on-farm study reported a statistical association between prevalence of *S. enterica* positive pigs and C&D procedures (van der Wolf *et al.*, 2001). In 353 sampled herds, those which “sometimes or always” used disinfectants after pressure washing a compartment were associated with a higher *S. enterica* seroprevalence than herds that “never” used a disinfectant after pressure washing (never disinfect OR=0.7, sometimes or always disinfect (reference category) OR=1). In this study, disinfecting procedures alone, rather than C & D procedures together, was associated with high *S. enterica* seroprevalence in pigs. As the authors noted, reliance on disinfectants may create a situation where producers perform inadequate cleaning prior to usage, whereas producers who realize disinfectants will not be applied may be more likely to perform a thorough job of cleaning. This could contribute to the counterintuitive association found in this study.

As with van der Wolf’s study, a case-control study by Stege (Stege *et al.*, 1997) found “manure-free cleaning between batches” and “disinfection between batches” positively associated with high seroprevalence of *S. enterica* in finishing pigs (OR=4.2 and OR=4.8 respectively). High-prevalence herds (n=39) included in this study had been previously identified as high-prevalence and confirmed to remain high. Low prevalence herds (n=69) were randomly selected based on historical seroprevalence data.

Bias, in particular confounding by indication, likely contributed to these counterintuitive results. Herds previously determined as “high” prevalence may have been more likely to adopt C & D procedures in an attempt to reduce seroprevalence, while “low” prevalence herds were unlikely to adopt new or improved C & D procedures. Rather than C & D causing “high” prevalence, it may be that “high” prevalence caused the adoption of C&D practices. Resulting association may seem spurious and are not causal.

Only one cohort study met inclusion criteria for this review (Funk *et al.*, 1999). Authors used increased fecal accumulation as a proxy variable for hygiene or lack of C & D procedures. An overall negative association between high levels of fecal accumulation and relative risk of a pig shedding *S. enterica* was reported in this study, i.e. pens with more fecal material tended to contain fewer pigs shedding *S. enterica*.

Transportation and lairage environment

Three cross-sectional studies (Bahnson and Fedorka-Cray, 1997, Bahnson *et al.*, 2001, Baum *et al.*, 1997) failed to identify a significant association between trailer hygiene and high prevalence of *S. enterica* in finishing pigs at slaughter.

The first study (Bahnson and Fedorka-Cray, 1997) was unable to find significant associations between either “gross contamination of truck” or “no sanitation of slaughter transport truck” and high prevalence of *S. enterica* in finishing pig fecal or tissue samples at harvest. Samples from 1057 pigs from 70 different farms were included in this study. A weak a sparing effect ($0.2 < p\text{-value} < 0.3$) of no sanitation of transport truck versus any sanitation method was found (OR=0.4). While limited by sample size, some inference was made regarding lack of association between herd size and presence of *S. enterica* on farms.

The second study also by Bahnson (Bahnson *et al.*, 2001) also failed to identify a significant association between variables involving sanitation of trailers prior to harvest transport and prevalence of *S. enterica* in finishing pig tissues at harvest. Fifty-one farms were included for analysis based on completed questionnaire data.

Baum et al (Baum *et al.*, 1997) examined transport level C & D in the same study which examined on-farm C & D procedures. C & D procedures defined as “cleaning trailers before hauling” had no significant association with seroprevalence of Salmonella in finishing pigs.

Discussion

Although these field trial studies differ in many ways, they have many similar problems which make interpretation of results difficult. For these studies, exposure status was determined by questionnaire allowing for possible recall bias. With recall bias, it is possible that producers responded with answers they felt researchers expected or which were more socially acceptable (Clarke *et al.*, 2003). Such actions may have biased study findings.

Generally, all studies appeared to be large with greater than 50 farms included, except for Quessy’s study (n= 28). However within farms, herd prevalence was often estimated utilizing very few samples (e.g. Quessy et al. n= 5, Beloeil et al. n=14). Given low in herd sampling numbers, confidence intervals around pen or herd prevalence estimates would be wide and contribute to a lack of statistical power because of measurement error. Therefore, lack of association in the included studies may be due to lack of statistical power rather than a true lack of difference. Another potential problem in these studies was the use of proxy variables which encompass many concurrent biosecurity or hygiene practices. Use of proxy variables makes it impossible to attribute associations to a single biosecurity or hygiene practice.

Section 4

Overall discussion and conclusions

It is doubtful that C & D procedures alone reduce the prevalence of *S. enterica* infected pigs in pre-harvest settings without concurrent implementation of other hygiene, biosecurity and/or management practices. Although experimental studies suggest disinfectants can reduce culturable *S. enterica* on material surfaces utilized in pre-harvest environments, the

vast majority of field trials and observational studies fail to find an association between reduced environmental prevalence of *S. enterica* and a reduced prevalence in pigs at harvest. It could however be argued that these studies suffer from biases, such as recall, selection, misclassification bias. Although taken as a body of work, the literature suggests that C & D procedures do not, by themselves, reduce *S. enterica* prevalence in pigs at harvest. Even the ability of disinfectants to reduce or eliminate residual *S. enterica* in pig housing and transport facilities is at best inconsistent due to extraneous factors which may influence the ability of C & D procedures to effectively eliminate culturable *S. enterica*.

Not only do results from numerous observational studies consistently suggest *S. enterica* is unlikely to be removed from pre-harvest facilities by cleaning and disinfecting, but our understanding of the ecology of *S. enterica* in its micro- and macroenvironment suggests a rational explanation. In the microenvironment of a biofilm community, bacteria develop increasing resistance to disinfectants through both intrinsic and extrinsic mechanisms. Starvation stress from low nutrient availability decreases uptake of chemicals into cells bacteria and alters metabolic pathways leading to decreasing susceptibility to disinfectants. Also, the organic nature of biofilm glycocalyx impedes the ability of many disinfectants penetrate to reach bacteria lying deep within the biofilm. The amount of biofilm glycocalyx increases in multi-species biofilms, which are commonly found in pig production environments. These multi-species biofilms also create more micro-niche environments which further decrease the ability of disinfectants to successfully reach and kill *S. enterica* organisms. To provide better biofilm penetration, employment of a cleaning step, either physically with high-pressure water or chemically with detergents, prior to disinfection removes upper layers of the biofilm. This step allows for greater access of disinfectants to deeper lying biofilm bacteria and increases efficacy of applied disinfectant products. Despite implementation of cleaning steps, residual bacteria are likely to remain.

In the macroenvironment of pig production, *S. enterica* is ubiquitous. Surfaces capable of disinfection are not the sole source of *S. enterica* exposure to pigs. Vermin, flies, other domestic animals, wildlife and contaminated feed supplies also serve as reservoirs for *S. enterica* on pig farms and contribute to re-contamination of disinfected facilities (Barber *et al.*, 2002, Harris *et al.*, 1997, Letellier *et al.*, 1999, Liebana *et al.*, 2003, Weigel *et al.*, 1999). Groups of pigs entering C & D facilities which include even one animal shedding *S. enterica* organisms can negate positive effects that *S. enterica*-free pens provide. This re-contamination by carrier animals becomes a major obstacle in the transport and lairage environment of pre-harvest production. Even sterile trailers or lairage pens cannot prevent shedding of *S. enterica* from pigs already infected with the organism and subsequent exposure of pigs in those environments. In reality, C & D procedures only attempt to prevent transmission of *S. enterica* from animals previously housed in facilities to those animals subsequently entering them. Unfortunately, C & D procedures cannot serve as a means of preventing pig to pig transmission, vermin to pig transmission or fomite to pig transmission of *S. enterica* organisms.

After reviewing literature addressing C & D procedures in the pre-harvest pig production environment, the importance of implementing those procedures should not be as a “silver bullet.” Since multiple sources of *S. enterica* exposure occur in the production environment, successful reductions in the pre-harvest prevalence of *S. enterica* in pigs will be maximized when C & D procedures are implemented as one part of an integrated hygiene and biosecurity program designed to reduce transmission of Salmonella from multiple reservoir sources.

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Table 2.1: Summary of included studies

Type of Study	Production Level	Conclusions	Source
experiment	laboratory	efficacy dependant on water temperature and type of disinfectant	El Assaad et al., 1990
experiment	laboratory	increasing efficacy with increasing concentration of chlorine on steel and plastic, minimal efficacy on wood	El Assaad et al., 1995
experiment	laboratory	3 of 13 compounds eliminated <i>S. enterica</i> , other 10 reduced numbers to varying degrees	Ramesh et al. 2002
experiment	laboratory	variable reduction in <i>S. enterica</i> biofilm with increasing concentration of halogen disinfectants and contact time - eliminations at highest concentration and contact time; consistent elimination of planktonic <i>S. enterica</i> cells at low concentration and low contact time	Joseph et al. 2001
descriptive	on-farm	reduced prevalence of <i>S. enterica</i> in "high" seroprevalence herds after implementation of consistent C & D procedures in conjunction with improved hygiene and management	Pedersen, 1997
descriptive	transport/lairage	ineffective in eliminating residual <i>S. enterica</i> contamination in trailer and lairage on 4 occasions	Oosterom et al. 1982
field trial	on-farm	ineffective in eliminating residual pen <i>S. enterica</i> contamination in 1 of 2 trials; lower fecal prevalence of <i>S. enterica</i> in C & D pens in 3 trials (2 statistically significant)	Oosterom et al. 1982
field trial	on-farm	no recoverable <i>S. enterica</i> , 85% prevalence of residual Enterobacteriaceae in pens; prevalence of <i>S. enterica</i> infection in finishing pigs 68% (treated) vs. 81% (control)	Oosterom, Notermans, 1983
field trial	on-farm	existing procedures reduced prevalence, improved procedures both reduced and increased prevalence in pens, pigs from improved pens had higher prevalence at slaughter (35% vs. 62%)	McLaren et al., 2001
field trial	on-farm	movement of <i>S. enterica</i> -free pigs into clean and disinfected facilities can prevent <i>S. enterica</i> infection in conjunction with other hygiene and biosecurity measures	Dahl et al., 1997
field trial	transport/lairage	no measurement of efficacy (prevalence) of cleaning and disinfection of trailers: no statistical difference in prevalence in pigs from treated or control trailers and lairage pens	Childers et al., 1977
field trial	transport/lairage	significant reduction in prevalence of <i>S. enterica</i> in trailer pens (41.5% to 2.7%), eliminated <i>S. enterica</i> in 94% of infected trailer pens	Rajkowski, et al., 1998
field trial	transport/lairage	ineffective in eliminating residual <i>S. enterica</i> in lairage pens, no pre-treatment measurement to assess ability to reduce prevalence, no significant difference in prevalence in pigs from treated or control lairage pens	Boes et al., 2001
field trial	transport/lairage	significantly reduce, but not eliminate <i>S. enterica</i> in lairage pens, improved procedures further reduce prevalence	Swanenburg et al., 2001a
field trial	transport/lairage	significantly reduce, but not eliminate <i>S. enterica</i> in lairage pens, <i>S. enterica</i> -free/sero-negative pigs contaminated with <i>S. enterica</i> between arrival to lairage and sampling post-slaughter although source of contamination not specifically attributed to sanitation failure	Swanenburg et al., 2001b

Table 2.1: Summary of included studies (cont.)

Type of Study	Production Level	Conclusions	Source
cross-sectional	on-farm	no association between cleaning and disinfection and incidence of fecal shedding of <i>S. enterica</i> in finishing pigs	Bush et. al., 1999
cross-sectional	on-farm	no association between cleaning and disinfection and incidence of fecal shedding of <i>S. enterica</i> in finishing pigs	Quessy et. al, 1999
cross-sectional	on-farm	no association between cleaning and disinfection and incidence of fecal shedding of <i>S. enterica</i> in finishing pigs	Beloeil et. al., 1997
cross-sectional	on-farm	no association between cleaning between groups or disinfecting between groups and seroprevalence of <i>S. enterica</i> antibodies in finishing swine	Baum et. al., 1997
cross-sectional	on-farm	positive association between disinfection after cleaning and seroprevalence of <i>S. enterica</i> antibodies	van der Wolf et.al., 2001
cross-sectional	transport/lairage	no association between cleaning trailers before hauling pigs and seroprevalence of <i>S. enterica</i> antibodies in finishing swine	Baum et. al., 1997
cross-sectional	transport/lairage	no association between trailer hygiene and <i>S. enterica</i> prevalence in caudal mesenteric lymph nodes at slaughter	Bahnson & Fedorka-Cray, 1997
cross-sectional	transport/lairage	no association between trailer hygiene and <i>S. enterica</i> prevalence in fecal samples or tissues from finishing pigs at slaughter	Bahnson et. al., 2001
case-control	on-farm	manure-free cleaning between batches (OR=4.2)and disinfection between batches (OR=4.8) associated with high seroprevalence of <i>S. enterica</i> in finishing pigs	Stege et. al., 1997
cohort	on-farm	positive association between "clean" pens and <i>S. enterica</i> infection in finishing pigs	Funk et. al., 2001

CHAPTER 3. THE ASSOCIATION BETWEEN CLEANING AND DISINFECTION OF LAIRAGE PENS AND THE PREVALENCE OF *SALMONELLA ENTERICA* IN SWINE AT HARVEST

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Abstract

A series of four field trials were conducted to evaluate the ability of a cleaning and disinfection procedure in swine lairage pens to reduce the prevalence of *S. enterica* in slaughtered pigs. A cleaning and disinfection procedure was applied to lairage pens at a large Midwest abattoir. Each trial consisted of a cleaned (alkaline chloride detergent) and disinfected (H₂O₂ + peracetic acid sanitizer) pen (treated) and a control pen, each holding 90-95 pigs for 2-3 hours before slaughter. Ileocecal lymph nodes, cecal contents, and rectal contents were collected from 45 pigs from each study pen at harvest and cultured for *S. enterica*. In all trials, cleaning and disinfection reduced the prevalence of *S. enterica* positive floor swabs in the treated pen ($p < 0.05$). However the post-harvest prevalence of *S. enterica* positive pigs varied between trials.

In Trial 1 there was no significant difference in the prevalence of *S. enterica* in pigs between treatment and control groups. In trials 2 and 3, the prevalence of *S. enterica* was

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higher in pigs from treated pens vs. pigs from control group pens (91% vs. 40%, $p < 0.0001$ and 91% vs. 24% $p < 0.0001$ respectively). In Trial 4 the prevalence of *S. enterica* was lower in pigs from treated pens compared to pigs from control pens (5% vs. 42%, $p < 0.0001$). This study indicates that cleaning and disinfection effectively reduces the amount of culturable *S. enterica* in lairage pens, but the ability of cleaned and disinfected pens to reduce the prevalence of *S. enterica* in market-weight pigs remains inconclusive.

Introduction

Several studies report a disparity between prevalence of *S. enterica* infected pigs on farm compared to pen mates slaughtered at the abattoir (5, 14, 15, 17). This disparity suggests that infection of swine with *S. enterica* may rapidly occur during transport from farm to abattoir and/or during holding of pigs in facilities at collection points or lairage pens at the abattoir. Furthermore, recent publications have indicated that market weight pigs can become infected with *S. enterica* typhimurium after exposure to a contaminated pen environment in 30 minutes (13).

Improving hygiene or sanitation remains a primary recommendation as a method of reducing pig exposure to *S. enterica* in the pre-harvest production environment (5, 12, 23, 26). Published studies about improved farm or lairage hygiene have reported a reduction in the prevalence of *S. enterica* in pigs (10, 21). Hygiene, however, represents a broad category of practices from cleaning and disinfection to all-in/all-out management to biosecurity measures. When these practices are implemented simultaneously, the impact of any single practice cannot be fully evaluated.

Given the current emphasis placed on identifying interventions to reduce the amount of *S. enterica* found in pork and the lack of conclusive results from previous studies, a series of field trials were designed to evaluate the ability of a cleaning and disinfection procedure in swine lairage pens to reduce the prevalence of *S. enterica* in slaughtered pigs. The rationale of the study being that reducing the quantity of *S. enterica* in the swine lairage pen available to rapidly infect swine would reduce the prevalence of *S. enterica* positive pigs at slaughter.

The null hypothesis was that pigs held in cleaned and disinfected lairage pens would have the same prevalence of culturable *S. enterica* in lymph node, cecal and fecal samples compared to pigs held in an uncleaned lairage pen. The alternate hypothesis was that pigs held in cleaned and disinfected lairage pens would have a different prevalence of culturable *S. enterica* in lymph node, cecal and fecal samples compared to pigs held in a dirty lairage pen.

Materials and Methods

Abattoir facilities. All trials were conducted at a commercial abattoir in the Midwest region of the United States. The annual plant kill is around 4.5 million hogs (17,000 daily) sourced from 2,500 farms in 4 states. Lairage floors and walls were concrete. All alleyways and scales leading to the study pens were rinsed with high-pressure cold water prior to entry of study pigs. Alleyways leading from study pens to the kill area were also rinsed immediately before study pigs entered them. Study pigs were killed immediately following the morning break to allow time for ante-mortem veterinary inspection and minimum study exposure times. The trials occurred on Tuesdays at three week intervals (except for a two week interval between Trials 3 & 4) from March to May 2003.

Study pigs. Study pigs were from one multi-site production system, which marketed more than 20,000 pigs annually. Pigs arrived to the abattoir between 8:00 – 8:30 am and were unloaded into a rinsed sorting pen. Randomization occurred by alternating 5-10 pigs from the sorting pen onto two scales for weighing. After weighing, the pigs were moved to the treated (cleaned and disinfected) or control pen. Time from unloading to placement into the study pens was 20 to 30 minutes in Trials 1, 2 and 4. Unexpected plant downtime occurred during Trial 3 forcing a two-hour holding time in the sorting pen. Holding times in study pens prior to slaughter ranged from two to three hours for all trials.

Lairage treatment. Cleaning and disinfection of the treated pen involved a five-step procedure. Step 1): high-pressure cold water rinse. Step 2): application of alkaline chloride detergent diluted to 1.2% concentration (pH 12.3, 2.5% available chlorine), minimum contact time of 10 minutes. Step 3): high-pressure cold water rinse. Step 4): application of

hydrogen peroxide (6.9%), peroxyacetic acid (4.4%), and octanoic acid (3.3%) sanitizer diluted to 3.1% concentration, minimum contact time of 10 minutes. Step 5): high-pressure cold water rinse. Products were applied with separate four-gallon backpack style sprayers. After cleaning and disinfection, no visible debris remained on pen floors or walls. The control pen was moistened with cold water to approximate the same moisture level as the treated pen before study pigs entering the pen.

Sample size. A total of 40 floor samples per pen and 45 sets of pig tissue samples per treatment were collected, processed and cultured in each trial. With 80% power, this study was able to detect a 28% difference in prevalence in pen samples and a 22% difference in prevalence in pig samples as statistically significant with $\alpha = 0.05$.

Pen sample collection. Prior to cleaning and disinfection floor samples were collected from the control and treated pens. A sterilized four inch by four inch gauze pad was held at arms length, dropped and allowed to “flutter” to the floor. Contact time with the floor ranged from five to nine minutes before gauze pads were collected with sterilized tweezers into sterilized bags. After cleaning and disinfection the sampling procedure was repeated in the treated pen. All samples were transported in a cooler to the National Animal Disease Center in Ames, IA for culture. Samples were immediately processed on arrival at the National Animal Disease Center.

Pen sample culture procedure. 25 ml of buffered peptone water was added to each sample bag containing pen swabs. Samples were stomached for 30 seconds at 230 rpm (Stomacher 400 Circulator; Seward Ltd., London, UK). For pre-enrichment, 10ml of solution was added to a bag containing 90ml of tetrathionate broth (TET) and 10ml of solution was added to a bag containing 90ml buffered peptone water (BPW). The TET and BPW were incubated at 37°C for 24 hrs. After incubation, 0.1ml from TET and BPW was transferred into 9.9ml Rappaport-Vassiliadis enrichment broth containing 0.001µg Novobiocin (RV1) and incubated at 42°C for 24 hours. A second pre-enrichment followed as 0.1ml from RV1 was transferred into another 9.9ml Rappaport-Vassiliadis enrichment broth (RV2) and incubated at 42°C for 24 hours. After the second pre-enrichment i.e. on day three

post collection, samples were tested for the presence of *S. enterica* antigen by antigen capture ELISA (Assurance Gold EIA Salmonella, BioControl). Samples were considered positive if the optical density was greater than 0.40. Positive samples were streaked onto xylose lysine tergitol agar plates and brilliant green sulphapyridine agar plates and incubated at 37°C for 24 hours. Suspect *S. enterica* colonies were selected and streaked onto Rombach agar for confirmation and incubated at 37°C for 24 hours.

Pig sample collection. Viscera from 45 of the 90-95 pigs in each study pen were collected at slaughter. From each pig, approximately one gram of ileocecal lymph node was collected, using sterile equipment, into sterilized bags. Approximately ten grams of contents from the cecum and ten grams of distal colon/rectum contents were collected using a scissors to cut a small hole in the viscera and milking the contents into a sterile bag. All samples were transported in a cooler to the National Animal Disease Center in Ames, IA for culture. Samples were processed 18 hours post collection.

Pig sample culture procedure. Ten grams of cecal contents were placed into each of two sterile bags; one bag containing 90ml of tetrathionate broth (TET) and one bag containing 90ml buffered peptone water (BPW) then incubated at 37°C for 24 hrs. The same procedure was repeated with ten g fecal (distal colon/rectum) contents. Ileocecal lymph nodes were rinsed in ethyl alcohol and flamed, then placed in sterilized filter bags. Samples were smashed with a rubber mallet. 25ml PBS was added and samples then stomached for 30 seconds at 230 rpm. Ten milliliters of the stomached solution was added to a bag containing 90ml of tetrathionate broth (TET) and a bag containing 90ml buffered peptone water (BPW) and incubated at 37°C for 24 hrs. After incubation, 0.1ml from TET and BPW was transferred into 9.9ml RV1 and incubated at 42°C for 24 hours. A second pre-enrichment followed as 0.1ml from RV1 was transferred into another 9.9ml RV2 and incubated at 42°C for 24 hours. Buffered peptone water samples were tested for the presence of *S. enterica* by antigen capture ELISA (Assurance Gold EIA Salmonella, BioControl). Samples were considered positive if the optical density was greater than 0.40. . Samples determined positive were streaked onto XLT4 and BGS agar plates. All TET samples were

streaked onto XLT4 and BGS agar plates. Plates were incubated at 37°C for 24 hours. Suspect *S. enterica* colonies were selected and streaked onto Rombach agar for confirmation and incubated at 37°C for 24 hours.

Analysis. Statistical analysis was performed using SAS 8.2 software (SAS Institute Inc., Cary, North Carolina). For each pen, the prevalence of *S. enterica* was the number of positive *S. enterica* swabs divided by the number of total pen swabs. A series of two by two contingency tables were constructed between before and after, before and control, and after and control pens and a two-tailed Fisher's exact test used to determine associations between pen prevalence and pen treatment. Pig prevalence was determined using the number of positive tissues samples divided by the total number of tissues collected for each tissue type. Any positive pig prevalence was calculated using the number of pigs with at least one tissue type positive divided by the total number of pigs sampled. Two by two contingency tables were constructed between treated and control pigs and a two-tailed Fisher's exact test used to determine associations between pig prevalence and pen treatment.

The data were modeled using an extension of the generalized linear model as described by Wolfinger and O'Connor (28) and implemented using the GLIMMIX macro in SAS. The outcome variable was dichotomous, i.e. *S. enterica* positive (yes/no). Explanatory variables included treatment (fixed effect) and trial (random effect) and a treatment*trial interaction term (random effect). If the interaction term was significant, the trials were treated as separate events and Fishers exact test used as the test for statistical association. The measure of association used was the odds ratio and 95% Wald confidence intervals are presented.

The odds ratio (OR) is a ratio of the odds of a *S. enterica* positive pig from a treated pen compared to the odds of a *S. enterica* positive pig from a control pen. For example, for lymph node samples in trial one the odds of a sample being *S. enterica* positive to *S. enterica* negative were 2:37 in the treated pen and 3:39 in the control pen therefore the OR = 0.7. Odds ratios are a measure of the intensity of the relationship between a risk factor (cleaning & disinfection) and an outcome (*S. enterica* infection). An OR = 1 indicates no relationship

between the factor and the outcome. An $OR < 1$ indicates the factor is associated with a lower incidence of the outcome, and an $OR > 1$ indicates the factor is associated with a higher incidence of the outcome.

Results

S. enterica was identified in control and before-treatment study pens in all four trials with prevalence ranging from 20-100% positive swabs (Table 1). The prevalence of *S. enterica* positive swabs in the after-treatment pens ranged from 0%-15% , a significant difference in prevalence from before-treatment in all trials (see table 1).

The treatment*trial interaction term was significant in the GLIMMIX model suggesting some unmeasured difference between trials. Therefore trials are presented separately.

Results from pig samples are summarized in Table 2. In Trial 1, no statistically significant difference in *S. enterica* prevalence between treated and control pigs existed in any collected tissues ($p = 0.16$ to 1.00). In Trials 2 and 3 a significantly higher prevalence of *S. enterica* positive samples in fecal, cecal or any sample occurred in pigs from the treated pen compared to the pigs from the control pen ($p < 0.0001$, $OR \gg 1$). In Trial 2, *S. enterica* prevalence in lymph node tissues did not differ between treatment and control pigs ($p = 1.00$). However in Trial 3, the prevalence of *S. enterica* positive lymph nodes was significantly higher in the treated group ($p = 0.001$). In Trial 4, the control pigs had a significantly higher prevalence of *S. enterica* in fecal, cecal or any sample compared to treated pigs ($p < 0.003$ to $p < 0.0001$). The prevalence of *S. enterica* in lymph node tissues did not vary between treatment and control pigs ($p = 0.49$). Odds ratios for the association between treatment and the prevalence of *S. enterica* in pig tissues in each trial are included, in Table 2.

Discussion

The results from the four trials were inconsistent, with all possible outcomes occurring; no difference in prevalence (null hypothesis), treated group with higher prevalence, and control group with higher prevalence. We had anticipated that cleaning and disinfection would be associated with no effect or a protective effect, as found in trial 1 and 4. The results are, however, consistent with a previous study where cleaning and disinfection in swine production facilities both increased and decreased the prevalence of *S. enterica* in pigs (18). Cleaning and disinfection of swine production facilities and *S. enterica* prevalence in slaughter pigs are reported as having no association (1, 2, 3, 4, 6, 8, 9, 22), a positive association i.e. cleaning increased the prevalence of *S. enterica* in slaughtered pigs (24, 27), or a negative association i.e. cleaning decreased *S. enterica* prevalence in slaughtered pigs (19, 20).

The most likely explanation for the variability of the association between cleaning and disinfection and *S. enterica* prevalence is that biases have distorted the outcomes of these studies. Many of the studies were case-control studies, case reports, or cross-sectional studies, so recall bias, selection bias, and uncontrolled confounding may explain differences in outcomes. We chose to conduct a series of field trials to reduce the impact of bias on our outcome. Field trials have been conducted and found no association between *S. enterica* prevalence and cleaning and disinfection but several confounders remained uncontrolled. We addressed many of these confounders in our field trials. For example, we collected pen samples to ensure *S. enterica* exposure occurred. Furthermore, confounders such as variable sources of pigs and variable employees or staff performing cleaning and disinfection in each trial were eliminated by careful study planning and design.

The four trials make up a larger multi-center trial and therefore are similar to a multi-center trial. Multi-center trials frequently experience differences in center results attributable to unknown confounders associated with the center. It is, however, the involvement of multiple centers which enhances the generalizability of the results. The heterogeneity of both

the study population (pigs) and the centers (lairage environment) more closely resemble how cleaning and disinfection will be utilized in the modern lairage environment.

Despite our success in demonstrating a reduction in *S. enterica* recovery from pen floors, we could not consistently reduce the prevalence of *S. enterica* in pigs. Several explanations exist for this. In our study, an unknown confounder was possibly present when each trial was conducted. Despite controlling for confounding using randomization and restriction (i.e. single source of pigs, a single abattoir and a single person applying disinfection), the unknown confounding variable, appears to have led to variability or imprecision in the results.

While pigs were randomized by small groups into either treatment or control pens, we question whether this achieved randomization of previously infected pigs between groups. For example, it has been suggested that *S. enterica* positive lymph nodes may be more representative of previous or on-farm infection rather than recent exposure in the lairage environment. Also, recent data indicates clustering of *S. enterica* shedding animals in finishing pens (Rostagno, USDA, unpublished data). In light of this, the *S. enterica* prevalence in lymph nodes in Trial 3 may indicate a lack of randomization of study pigs (22% positive treatment pigs & 0% positive control pigs). The counterpoint to that observation is then that Trials 1, 2 and 4 would appear to have achieved adequate randomization (no difference in LN prevalence of *S. enterica* between groups).

One confounder may be the population of *S. enterica* present on the floor before and after disinfection. The genus *S. enterica* contains over 2400 serovars, which vary in environmental survivability, antimicrobial resistance, virulence, and pathogenicity. This diversity allows for existence of microcosms of organisms within the environment with variable phenotypic traits. In biofilm microcosms for example, *S. enterica* organisms existing close to the biofilm-solid surface interface have demonstrated increased resistance to antimicrobial agents (16) and increased virulence (7). The decreased ability of disinfectant agents to reach deep areas in biofilms (11, 25) may leave sub-populations of *S. enterica* in the newly cleaned environment which are more able to invade and/or infect susceptible

animals. Perhaps cleaning and disinfection left such a sub-population of *S. enterica* on the floor in Trail 2 and 3 which was more readily able to infect pigs. In Trial 4, cleaning and disinfections removed all culturable *S. enterica*; therefore the pigs did not become infected by a sub population of *S. enterica*.

The results of the current study, while inconsistent, highlight the lack of knowledge of the ecology of *S. enterica* in the abattoir. Although cleaning and disinfection can successfully reduce or eliminate *S. enterica* from lairage pens, consistent and significant reduction in the levels of *S. enterica* recovered from pigs held in those pens was not attained. Knowledge of the mechanisms involved in survivability of recovered *S. enterica* strains and increased virulence of biofilm strains may explain the results in this and previously studies. The results also illustrate the need to ensure a relevant outcome is measured during trials. If only the floor samples had been collected we may have falsely concluded, based on biological feasibility, that the prevalence in swine was likely to have decreased. By concentrating on the outcome of interest, the prevalence in pigs this error was avoided.

This study demonstrates that simple cleaning and disinfection of lairage pens is not a feasible intervention method for reducing the post-harvest prevalence of *S. enterica* in pigs in the modern lairage environment. Therefore use of cleaning and disinfection procedures should be as one part of a multi-step program applied from pre- to post-harvest pork production. Such a multi-step program may make an impact of the magnitude necessary to reduce the incidence of food borne Salmonellosis attributable to pork.

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Tables

Table 3.1: Frequency count of *S. enterica* positive samples in control pens, treated pens before cleaning and disinfection, and treated pens after cleaning and disinfection.

	Control (%)	Treated	
		Before (%)	After (%)
Trial 1	13/40 (33) ^a	8/40 (20) ^a	2/40 (5) ^b
Trial 2	29/40 (73) ^a	40/40 (100) ^b	2/40 (5) ^c
Trial 3	37/40 (93) ^a	40/40 (100) ^a	6/40 (15) ^b
Trial 4	40/40 (100) ^a	34/40 (85) ^b	0/40 (0) ^c

Within each row, results without a common superscript differ (p<0.05)

Table 3.2: *S. enterica* positive samples in cecal, fecal and ileocecal lymph node, and in at least one (any) samples from pigs held in treated and control pens. Odds ratios (OR) were not available (n/a) when the number of *S. enterica* positive samples was zero in at least one cell.

	Sample	Treated (%)	Control (%)	p-value	OR	(95% CI)
Trial 1	Cecal	0/39 (0)	3/42 (7)	0.24	n/a	
	Fecal	2/21 (10)	0/31 (0)	0.16	n/a	
	LN	2/39 (5)	3/42 (7)	1.00	0.7	(0.11-4.45)
	Any Sample	3/39 (8)	6/42 (14)	0.48	0.5	(0.12-2.15)
Trial 2	Cecal	31/45 (69)	3/45 (7)	<0.0001	31	(8.2-117.3)
	Fecal	33/38 (87)	3/33 (9)	<0.0001	66	(14.5-300)
	LN	15/44 (34)	16/45 (36)	1.00	0.94	(0.39-2.24)
	Any Sample	41/45 (91)	18/45 (40)	<0.0001	15.4	(4.7-50.4)
Trial 3	Cecal	38/45 (84)	10/45 (22)	<0.0001	19	(6.5-55.4)
	Fecal	18/36 (50)	2/30 (7)	<0.0001	14	(2.9-67.7)
	LN	10/45 (22)	0/45 (0)	0.001	n/a	
	Any Sample	41/45 (91)	11/45 (24)	<0.0001	31.7	(9.2-108.5)
Trial 4	Cecal	2/44 (5)	12/45 (27)	0.007	0.13	(0.3-0.62)
	Fecal	0/36 (0)	10/43 (23)	0.0015	n/a	
	LN	0/44 (0)	2/45 (4)	0.49	n/a	
	Any Sample	2/44 (5)	19/45 (42)	<0.0001	0.065	(0.01-0.3)

CHAPTER 4. ANTIMICROBIAL RESISTANCE PATTERNS OF *SALMONELLA ENTERICA* IN SWINE LAIRAGE PENS BEFORE AND AFTER PEN CLEANING AND DISINFECTING

A paper to be submitted to Veterinary Microbiology

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Abstract

Problem addressed: Cleaning and disinfecting (C & D) animal holding areas is commonly recommended, however the effects of these procedures on the antimicrobial resistance of residual *Salmonella enterica* populations needs to be addressed.

Objective: The aim of this study was to determine if C & D procedures changed the diversity of antimicrobial susceptibility patterns of *S. enterica* in swine lairage pens.

Methods and approach: Pen swabs were collected from an abattoir lairage pen on four separate days. A cleaning and disinfecting procedure was applied to one pen and the floor sampled again. *S. enterica* positive swabs were identified by antigen-capture ELISA (BioRad) following enrichment then streaked onto XLT and BGS agar plates. Antimicrobial resistance was determined using Kirby-Bauer disk diffusion test methods. Results were reported as sensitive, intermediate or resistant to tested antimicrobials. The antimicrobial

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resistance patterns of the isolates were determined by K-means clustering and visualized with Ggobi multidimensional visualization software.

Results: 132 of 320 pen floor swabs were determined to be positive for *S. enterica* with 123 isolates recovered as isolated colonies. The 123 isolates clustered into 10 resistance patterns. Prevalence of *S. enterica* positive pen swabs and resistance pattern diversity decreased after C & D procedures. C & D procedures appeared to be non-selective in overall residual *S. enterica* resistance patterns.

Conclusions: C & D procedures in swine lairage pens did not eliminate all culturable *S. enterica* from the pen environment nor did procedures select for a particular antimicrobial resistance pattern.

Introduction

Salmonella enterica has been implicated in numerous foodborne outbreaks associated with consumption of pork products (Gessner et al., 1994; Maguire et al., 1993; Narain et al., 1989). Costs associated with foodborne Salmonellosis are estimated between \$0.5 and \$2.8 billion annually in the United States alone (Frenzen et al., 1999). Recently, 3.3 - 16% of pork products from retail outlets and 5.8% of ground pork products from processing plants were found to be contaminated with *S. enterica* (Duffy et al., 2001; White et al., 2001; Zhao et al., 2001). Given the incidence of *S. enterica* contamination in pork products and costs associated with foodborne Salmonellosis, development and implementation of intervention methods to reduce the burden of *S. enterica* has become a priority in pre-, peri- and post-harvest sectors of pork production.

Cleaning and disinfecting animal facilities is one of the most commonly recommended intervention methods designed to reduce *S. enterica* in the pre-harvest pork production environment. However, the excessive use of disinfecting agents has been criticized in the public domain and medical community due to concern that widespread use of disinfectants may lead to increase bacterial resistance, in a manner similar to use of antimicrobial agents. These concerns are based on experimental data which suggests that bacteria in biofilms

develop resistance to disinfectants (Gilbert et al., 2001; Oosthuizen et al., 2002; Tremoulet et al., 2002) and studies that have found associations between the use of disinfectants in hospitals and biocide resistance in bacteria (Schwartz et al., 2003) Therefore, we assessed the antimicrobial resistance patterns of *S. enterica* isolates collected during a clinical trial designed to assess the efficacy of cleaning and disinfecting in swine lairage pens. The purpose of this report was to describe the antimicrobial resistance patterns of *S. enterica* present on swine lairage pen floors before and after application of a cleaning and disinfecting protocol.

Materials and Methods

Lairage facilities

This field trial was conducted at a commercial abattoir in the Midwest region of the United States. The annual plant kill is approximately 4.5 million hogs (17,000 daily) sourced from 2,500 farms in four states. Lairage floors and walls were concrete. Pens were sampled at three-week intervals (except for a two week interval between pens three & four) from March to May 2003.

Cleaning and disinfecting procedures

Cleaning and disinfecting of the lairage pen involved a five-step procedure. Step one): high-pressure cold water rinse. Step two): application of alkaline chloride detergent diluted to 1.2% concentration (pH 12.3, 2.5% available chlorine), minimum contact time of 10 minutes. Step three): high-pressure cold water rinse. Step four): application of hydrogen peroxide (6.9%), peroxyacetic acid (4.4%), and octanoic acid (3.3%) sanitizer diluted to 3.1% concentration, minimum contact time of 10 minutes. Step five): high-pressure cold water rinse. After cleaning and disinfecting, no visible debris remained on pen floors or walls.

Sample collection

Prior to cleaning and disinfecting, 40 floor samples were collected. Samples consisted of sterilized four inch by four inch gauze pad held at arms length, dropped, and allowed to “flutter” to the floor. Contact time with the floor ranged from five to nine minutes before gauze pads were collected with sterilized tweezers and placed in sterilized bags. After cleaning and disinfecting, the sampling procedure was repeated with 40 additional gauze pads. All samples were transported in a cooler to the National Animal Disease Center in Ames, Iowa and processed immediately on arrival.

S. enterica isolation and selection procedures

Twenty-five milliliters of buffered peptone water was added to each sample bag containing pen swabs. Samples were stomached for 30 seconds at 230 rpm (Stomacher 400 Circulator; Seward Ltd., London, UK). For pre-enrichment, 10ml of solution was added to a bag containing 90ml of tetrathionate broth (TET) and 10ml of solution was added to a bag containing 90ml buffered peptone water (BPW). The TET and BPW were incubated at 37°C for 24 hrs. After incubation, 0.1ml from TET and BPW was transferred into 9.9ml Rappaport-Vassiliadis enrichment broth containing 0.001µg Novobiocin (RV1) and incubated at 42°C for 24 hours.

A second pre-enrichment followed as 0.1ml from RV1 was transferred into another 9.9ml Rappaport-Vassiliadis enrichment broth (RV2) and incubated at 42°C for 24 hours. After the second pre-enrichment i.e. on day three post collection, samples were tested for the presence of *S. enterica* antigen by antigen capture ELISA (Assurance Gold EIA Salmonella, BioControl). Samples were consider positive if the optical density was greater than 0.40. Positive samples were streaked onto xylose lysine tergitol agar plates (XLT) and brilliant green sulphapyridine agar (BGS) plates and incubated at 37°C for 24 hours, then set at room temperature for 42 hours. Therefore, 3 days after collection, each sample was growing on four plates, TET-XLT, TET-BGS, BPW-XLT, and BPW-BGS. Suspect *S. enterica* colonies

were selected and streaked onto Rombach agar for confirmation and incubated at 37°C for 24 hours.

Antimicrobial resistance procedures

A single colony from the TET-XLT4 plate was selected for antimicrobial susceptibility testing. If no growth occurred on the TET-XLT4 plate, a colony was selected from the TET-BGS plate. Again if no growth was present on either TET plate, a colony was selected from the BPW-XLT4 plate and finally from the BPW-BGS plate if no growth occurred on the three previous plates. Kirby-Bauer antimicrobial disk susceptibility (antibiogram) tests were performed in accordance with National Committee for Clinical Laboratory Standards (NCCLS) Document M31-A2 (Anonymous, 2002). Antimicrobial disks (with concentration) included: amikacin (30 µg), ampicillin (10 µg), apramycin (15 µg), ceftiofur (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), florfenicol (30 µg), gentamicin (10 µg), streptomycin (10 µg), sulfamethoxazole (23.75 µg)/trimethoprim (1.25 µg), tetracycline (30 µg) (BBL™ Sensi-Disc™, Becton Dickinson and Company, Sparks, MD). Antimicrobial disk diameters were measured and classified as sensitive, intermediate, or resistant according to NCCLS guidelines (Anonymous, 2002). For antibiotics without NCCLS published animal breakpoints (ceftriaxone and streptomycin), the BBL™ Sensi-Disc™ package insert human breakpoint values were used (Table 1).

Statistical analysis

Statistical analyses were performed using JMP 5.1 software (SAS Institute Inc., Cary, North Carolina). Reductions in pen prevalence of *S. enterica* positive swabs were determined as significant using the Fisher's exact test, the null hypothesis being no difference in pen prevalence of *S. enterica* positive pens swabs following pen cleaning and disinfecting. Antibiogram patterns of *S. enterica* isolates were clustered using K-means algorithms in JMP5.1 software with a discrete resistance variable (sensitive, intermediate or resistant) and initial seed of 100 clusters. Pattern clusters were then visualized by combining 2-D touring

and parallel coordinates plots in Ggobi software (www.ggobi.org). Ggobi software allows the user to enter the resistance patterns of all individual isolates and examined the data visually in a multi-dimensional format to determine where patterns were similar.

Results

Cleaning and disinfecting procedures consistently reduced the prevalence of *S. enterica* positive floor samples (Table 2). Only in pen 4 was *S. enterica* prevalence reduced to zero following pen cleaning and disinfecting.

S. enterica was identified by ELISA and/or culture methods in 132 of 320 pen floor swabs (Table 2). One hundred and twenty-three *S. enterica* isolates were selected as isolated colonies from XLT or BGS agar plates (nine samples were positive by ELISA but had no growth with culture methods); 114 from pens before cleaning and disinfecting, 9 from pens after cleaning and disinfecting. The recovered isolates clustered into 10 antibiogram patterns (Table 3). Before disinfection 1 to 6 antibiogram patterns were found in each lairage pen. After disinfection, 0 to 2 antibiogram patterns were recovered from lairage pens. In 2 of 4 pens, at least one antibiogram cluster identified after disinfection was not identified in the pen before cleaning and disinfecting procedures (Table 4).

S. enterica isolates exhibited the greatest degree of resistance to spectinomycin and tetracycline with 86 (70%) and 54 (44%) resistant isolates respectively (Table 3). None of the 123 isolates displayed resistance to amikacin, apramycin, ciprofloxacin, gentamicin or sulfa/trimethoprim. Thirty-five (28%) of all isolates displayed no resistance to any of the 12 tested antimicrobials, 33 (30%) before isolates and two (22%) after isolates. Common multi-antimicrobial resistance combinations included spectinomycin plus tetracycline (ST) with seven (6%) resistant isolates. Other common resistance combinations include ST plus ampicillin (AST) and AST plus florfenicol (AFST) each with 1 (1%) and 11 (9%) resistant isolates respectively. A more common combination in this study was AST plus a 3rd generation cephalosporin (ceftiofur or cephalothin) (AST3) with 27 (22%) of total isolates. Resistance to 1 or more antimicrobials occurred in 88 (72%) isolates; 81 (71%) before and 7

(78%) after isolates (Table 5). Only 52 (42%) total isolates were resistant to 2 or more antimicrobials; 47 (41%) before and 5 (56%) after isolate (Table 5). Penta-resistant patterns were exhibited by 36 (29%) of total isolates; 32 (28%) before and 4 (44%) after isolates. No isolates exhibited resistance to seven or more tested antimicrobials.

Discussion

The goal of pre-harvest intervention strategies, such as lairage pen cleaning and disinfecting, is to reduce or eliminate exposure of swine to *S. enterica* in the pre-harvest environment and therefore reduce *S. enterica* in swine at harvest. While achieving this goal, we were also concerned about the effects of the lairage pen cleaning and disinfecting on the residual bacterial populations. In areas where disinfectants are used commonly and/or in high concentration, such as hospitals, there is growing concern that disinfectant use may select for more resistant, and possibly more virulent, bacterial strains.

The role of plasmid transfer of resistance to both disinfectants and antimicrobials in bacteria is minimal (Russell et al., 2000; Pearce et al., 1999). In our setting we did not realistically expect that a single application of disinfectants could induce changes in the bacterial population through mechanisms such as plasmid transfer. Of more concern in the lairage setting is the role of phenotypic changes in the disinfectant and antimicrobial resistance patterns of biofilm cells (Dhir et al., 1995; Elvers et al., 2002). Biofilms occur as bacteria naturally adhere to solid surfaces and form a layer of bacteria, extracellular polysaccharide, and debris. As cells age within a biofilm, they move farther from the air or liquid interface and therefore farther from available nutrients (Carpentier and Cerf, 1993). To cope with the reduced nutrient availability the bacteria in the inner layers of the biofilm undergo starvation induced phenotypic changes. These starvation-induced phenotypic changes also increase inherent resistance to disinfectants and antimicrobials (Gilbert et al., 2001; Oosthuizen et al., 2002; Tremoulet et al., 2002). Additionally, similar survival mechanisms may increase virulence of deep biofilm bacteria as well (Bonafonte et al., 2000). Therefore our concern was that the application of cleaning and disinfecting protocols in

swine lairage pens could disrupt and remove surface biofilm bacteria leaving more resistant and possibly more virulent bacteria exposed to incoming swine.

In this study, the number of antibiogram clusters present on lairage pen floors was reduced by cleaning and disinfecting procedures. These procedures, however, appeared to be non-selective overall with regards to the antibiogram patterns of the remaining isolates with no consistency in the patterns of isolates recovered after pen cleaning and disinfecting. This overall conclusion of non-selective disinfection process agrees with other published work that failed to demonstrate a higher degree of disinfectant resistance in antibiotic-resistance hospital isolates versus antibiotic-susceptible non-hospital isolates (Rutala et al., 1997). Our results and those of Rutala (Rutala et al., 1997) do not agree, however, with the results of Schwartz (Schwartz et al., 2003) where correlations existed between antibiotic-resistance and disinfectant-resistance. Schwartz examined antimicrobial resistance in bacterial isolates from water samples which may have been exposed to multiple disinfectant products and numerous species of bacteria. These factors may have contributed to increased levels of resistance in recovered isolates. Our study involved examination of isolates exposed to a single cleaning and disinfection protocol followed by immediate sampling. There was no time for a build-up in resistance or exposure to other remaining bacterial species.

While this apparent non-selective property of lairage pen cleaning and disinfecting adds to the potential benefits of implementing these procedures as part of a pre-harvest *S. enterica* intervention program, selection of more virulent residual organisms could negate those benefits. Unfortunately, virulence of remaining *S. enterica* isolates was not investigated in this study so no conclusions may be drawn regarding possible effects of lairage pen cleaning and disinfecting on isolate virulence.

The resistance patterns and prevalence of resistance to specific antibiotics that we report were similar to those that have been reported previously in US based swine production systems (Farrington et. al., 2001; Gebreyes et al., 2000; Gebreyes et al., 2003). This suggests that the bacteria at the lairage are representative of the production units that supply swine to the lairage.

A drawback in interpretation of results in this study is the use of categories to assign resistance to the bacteria. Categorizing bacteria as resistant, susceptible, and intermediate is a crude method of phenotyping and it is possible that differences in phenotypic resistance do actually exist but using this method changes could not be detected. For example, it is possible that the post- disinfecting bacteria were more resistance to the antibiotics measured, but they still were below the “cutoffs” used. In this way resistance can be “creeping” up closer to the break point but go undetected. To detect this type of resistance “creep”, a titration-based system or the exact zonal distance of inhibition using the KB methods should be reported and assessed.

A second drawback in interpretation of results in this and other studies is the lack of veterinary-specific breakpoints for enterica or non-clinical *S. enterica* in swine and lack of human breakpoints for enteric disease caused by *S. enterica*. Breakpoints can be interpreted as the probability of clinical efficacy of the antimicrobial in a specific species with a specific pathogen causing specific symptoms (Apley, 2003). Generalizing these breakpoints to other species, pathogens, or disease syndromes may not be indicative of probable clinical efficacy. In the case of enteric Salmonellosis caused by *S. enterica* from pork, breakpoints reported in this study cannot be interpreted as probability of clinical success in treating individuals infected with any of the recovered strains.

Conclusion

In the current study, cleaning and disinfecting procedures in swine lairage pens reduced, but did not eliminate all culturable *S. enterica* from the pen environment. In addition, cleaning and disinfecting procedures appeared to be non-selective in remaining antimicrobial resistance patterns.

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Tables

Table 4.1: Antimicrobial disks used to determine the phenotypic resistance patterns of 123 *Salmonella enterica* isolates collected from four lairage pens from March - May, 2003: antimicrobial agent, disk concentration (μg), and diameter (mm) of breakpoint cutoffs.

Antimicrobial Agent	Disk Content (μg)	Zone Diameter (mm)		
		S	I	R
Amikacin	30	≥ 17	15-16	≤ 14
Ampicillin	10	≥ 17	14-16	≤ 13
Apramycin	15	≥ 15	12-14	≤ 11
Ceftiofur	30	≥ 21	18-20	≤ 17
Ceftriaxone	30	≥ 21	14-20	≤ 13
Cephalothin	30	≥ 18	15-17	≤ 14
Ciprofloxacin	5	≥ 21	16-20	≤ 15
Florfenicol	30	≥ 19	15-18	≤ 14
Gentamicin	10	≥ 15	13-14	≤ 12
Streptomycin	10	≥ 15	12-14	≤ 11
Sulfamethoxazole /Trimethoprim	23.75 / 1.25	≥ 16	11-15	≤ 10
Tetracycline	30	≥ 19	15-18	≤ 14

Table 4.2: Prevalence of *Salmonella enterica* pen swabs collected from four lairage pens from March – May, 2003 before and after lairage pen cleaning and disinfecting.

	Prevalence of positive pen swabs (%)		
	Before	After	p-value
Overall	122/160 (76)	10/160 (6)	<0.001
Pen 1	8/40 (20)	2/40 (5)	0.09
Pen 2	40/40 (100)	2/40 (5)	<0.001
Pen 3	40/40 (100)	6/40 (15)	<0.001
Pen 4	34/40 (85)	0/40 (0)	<0.001

Table 4.3: Antibio gram pattern clusters, percentage of isolates in each cluster and percentage of isolates with individual antimicrobial resistance of 123 *Salmonella enterica* isolates collected from four lairage pens from March - May, 2003

Cluster ID	Amikacin	Ampicillin	Apramycin	Ceftiofur	Ceftioxone	Cephalothin	Ciprofloxacin	Flortenicol	Gentamicin	Streptomycin	Sulfamethoxazole / Trimethoprim	Tetracycline	total isolates n (%)
1	S	S	S	S	S	S	S	S	S	S	S	S	35 (28.5)
2	S	S	S	S	S	S	S	S	S	R	S	S	34 (27.7)
3	S	S	S	S	S	S	S	S	S	S	S	R	2 (1.6)
4	S	S	S	S	S	S	S	S	S	R	S	R	8 (6.5)
5	S	R	S	S	S	S	S	S	S	R	S	R	1 (0.8)
6	S	R	S	S	S	S	S	R	S	R	S	R	11 (8.9)
7	S	R	S	I	I	R	S	R	S	R	S	R	5 (4.1)
8	S	R	S	R	S	R	S	R	S	R	S	R	1 (0.8)
9	S	R	S	R	I	R	S	R	S	R	S	R	25 (20.3)
10	S	R	S	I	R	R	S	R	S	R	S	R	1 (0.8)
Susceptible n (%)	123 (100)	79 (64)	123 (100)	91 (74)	92 (75)	91 (74)	123 (100)	80 (65)	123 (100)	37 (30)	123 (100)	69 (56)	
Intermediate n (%)	0 (0)	0 (0)	0 (0)	6 (5)	30 (24)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Resistant n (%)	0 (0)	44 (36)	0 (0)	26 (21)	1 (1)	32 (26)	0 (0)	43 (35)	0 (0)	86 (70)	0 (0)	54 (44)	

Table 4.4: The distribution of the antibiogram patterns of 123 *Salmonella enterica* isolates collected before and after cleaning and disinfecting from four lairage pens from March – May, 2003. Cluster characteristics are described in Table 4.3.

Pen	Before Antibiogram Cluster ID	After Antibiogram Cluster ID
1	1, 3, 4	1, 5
2	3, 6, 7, 8, 9, 10,	1, 9
3	2, 4, 6	2, 6
4	1	none
Overall	1, 2, 3, 4, 6, 7, 8, 9, 10	1, 2, 5, 6, 9

Table 4.5: The frequency and percentage of 114 before, 9 after and 123 total *Salmonella enterica* isolates exhibiting multiple drug resistance patterns before and after cleaning and disinfecting collected from four lairage pens from March to May, 2003.

	Before		After		Total	
	N = 114		N = 9		N = 123	
Resistance to:	n.	%	n.	%	n.	%
ST	7	6	0	0	7	6
AST	0	0	1	11	1	1
AFST	8	7	3	33	11	9
AST3	26	23	1	11	27	22
No antimicrobials	33	29	2	22	35	28
1 or more	81	71	7	78	88	72
2 or more	47	41	5	56	52	42
5 or more	32	28	4	44	36	29
7 or more	0	0	0	0	0	0

A=ampicillin, F=florfenicol, S=streptomycin, T=tetracycline, 3=3rd generation cephalosporin
For analysis, antibiograms were dichotomized with intermediate categorized as susceptible

CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

The main objective of this project was to determine the applicability of lairage pen cleaning and disinfecting as an intervention method for reducing the prevalence of *S. enterica* in market swine. In order to better address such a large goal, the main objective was further divided into three sub-objectives.

1. Does thorough cleaning and disinfecting reduce or eliminate the prevalence of *S. enterica* organisms present in the lairage pen?
2. Does thorough cleaning and disinfecting result in a lower prevalence of *S. enterica* infected pigs when held in cleaned and disinfected rather than unclean pens?
3. Do the antibiogram resistance patterns of recovered lairage pen *S. enterica* isolates differ before and after application of pen cleaning and disinfecting?

This project has successfully answered all three sub-objective questions, although the answers were not always what had been anticipated.

Does thorough cleaning and disinfecting reduce or eliminate the prevalence of S. enterica organisms present in the lairage pen?

Utilization of a thorough cleaning and disinfecting protocol in this project successfully reduced the prevalence of *S. enterica* positive floor swabs in all four trials. This finding was consistent with previously published research (McLaren *et al.* 2001, Rajkowski *et al.* 1998, Swanenburg *et al.* 2001). The inability of cleaning and disinfection procedures to consistently eliminate culturable *S. enterica* organisms was also consistent between results of the current project and previously published research (Boes *et al.* 2001, El Assad *et al.* 1995, Oosterom *et al.* 1982, Ramesh *et al.* 2002). Only trial four in the current project yielded no culturable *S. enterica* isolates following pen cleaning and disinfecting. This cannot however be interpreted as trial four resulted in a pen free of *S. enterica*. Sampling error and/or culture error may have contributed to the collection of 40 after samples from

which no *S. enterica* organisms could be identified. The results of the current project, in conjunction with the results of previous experimental and field trial studies, indicate that cleaning and disinfection procedures can successfully reduce the prevalence of *S. enterica* organisms in swine lairage pens.

Does thorough cleaning and disinfecting result in a lower prevalence of S. enterica infected pigs when held in cleaned and disinfected rather than unclean pens?

Previously published research has not successfully answered this question with a large degree of variation in the association between cleaning and disinfecting and *S. enteric* prevalence in pigs (Funk *et al.* 2001, McLaren *et al.* 2001, Pedersen 1997, Stege *et al.* 1997, Swanenburg *et al.* 2001, van der Wolf *et al.* 2001). As stated in Chapter 3, the variability of the association between cleaning and disinfection and *S. enterica* prevalence in pigs may be partially explained by biases which have distorted the outcomes of these studies. Many studies were case-control studies, case reports or cross-sectional studies, so recall bias, selection bias and uncontrolled confounding may explain differences in outcomes. Field trials have been conducted and found no association between *S. enterica* prevalence and cleaning and disinfection but several confounders remained uncontrolled.

Having reduced the possibility of bias and confounding through careful study design, the current project provided unanticipated answers to the question of pen cleaning and disinfecting and *S. enterica* in pigs. In four field trials, all three possible associations occurred; higher, lower, and no difference in prevalence of *S. enterica* infected pigs between cleaned and not cleaned pens. These results highlight the fact that much of the ecology of *S. enterica* in the lairage environment is yet unknown. Unknown confounding variables may have contributed to the convoluted study results although randomization of pigs and the multi-centric nature of the study design should have minimized such confounding. Similarly, the relationship between *S. enterica* and pigs has not been fully elucidated and may have contributed the study results. Further analysis of *S. enterica* isolates recovered from both

pens and pigs may shed light on the inability of lairage pen cleaning and disinfecting to consistently reduce the prevalence of *S. enterica* infected pigs.

Do the antibiogram resistance patterns of recovered lairage pen S. enterica isolates differ before and after application of pen cleaning and disinfecting?

The resistance patterns and prevalence of resistance to specific antibiotics that we report were similar to those that have been reported previously in US based swine production systems (Farrington et. al., 2001; Gebreyes et al., 2000; Gebreyes et al., 2003) and in similar lairage prevalence research involving cull sows (Larsen, Iowa State University, unpublished). Other than an increase in the prevalence of streptomycin/tetracycline resistant *S. enterica* isolates, no significant shift in antimicrobial resistance occurred after lairage pen cleaning and disinfecting. This apparent non-selective property of lairage pen cleaning and disinfecting adds to the potential benefits of implementing such procedures in an effort to reduce *S. enterica* contamination in lairage environments. However, yet unanswered questions regarding the long term implications of plasmid transfer of antimicrobial and disinfectant resistance as well as selection for more virulent *S. enterica* organisms still need to be addressed before such programs are routinely implemented in the lairage setting.

Lairage pen cleaning and disinfecting shows promise as an intervention tool to reduce the environmental burden of *S. enterica* in the pre-harvest setting. Although elimination of all culturable organisms is rare, consistent reductions in the prevalence of culturable *S. enterica* organisms will decrease the burden of exposure to pigs held in those pens. Until the ecology of *S. enterica* in pigs is fully elucidated however, use of lairage pen cleaning and disinfecting would not be the intervention tool of choice.

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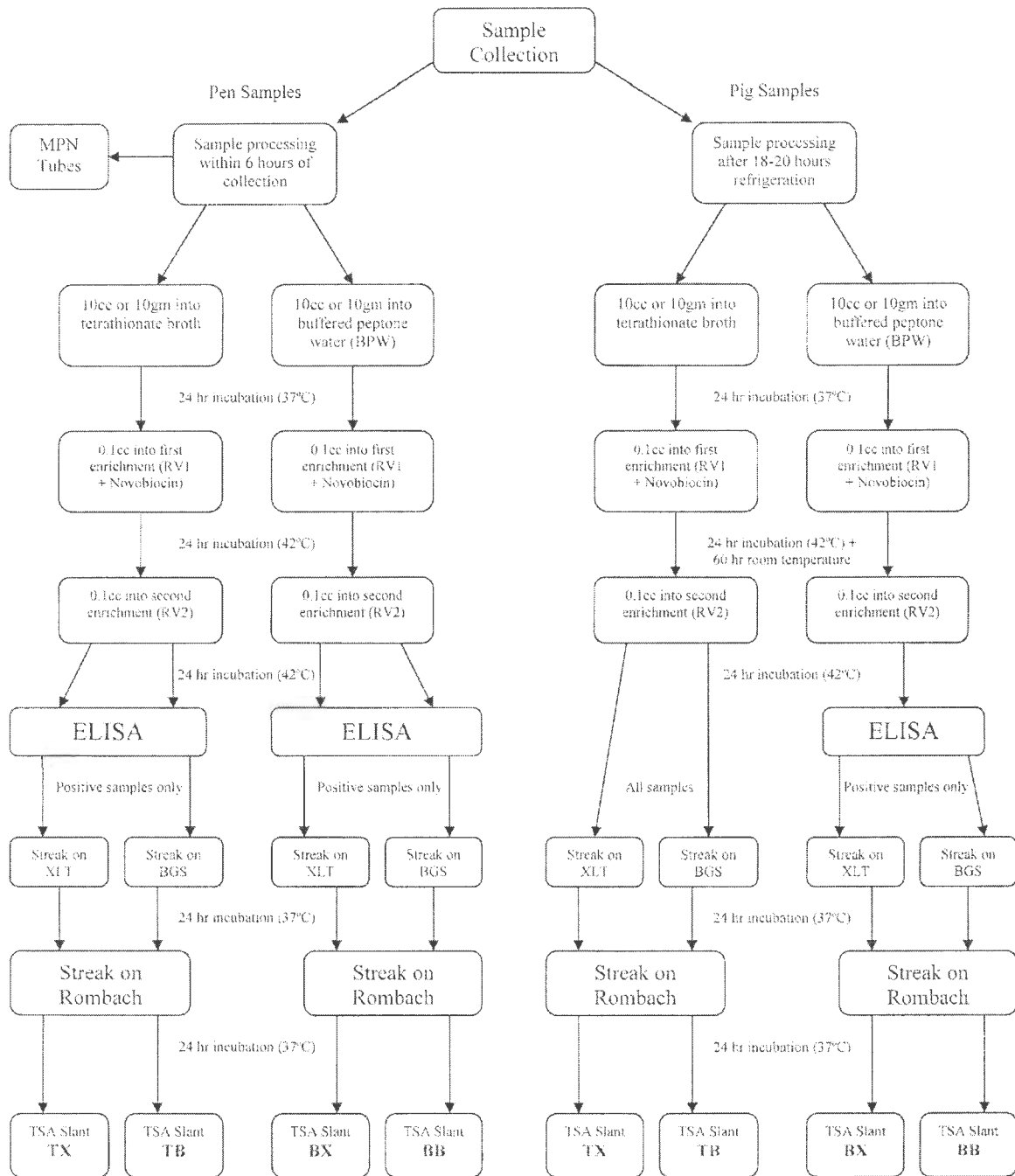
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**APPENDIX. FLOW CHART DIAGRAM OF *S. ENTERICA*
IDENTIFICATION PROTOCOL**

Protocol for identification of *S. enterica* organisms used in current project and developed in lab B-14; USDA, Animal Research Service, National Animal Disease Center, Ames, IA.



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